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NUTRITIONAL ECOLOGY OF NUISANCE AQUATIC PLANTS

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Three procedures were developed to evaluate the capacities of macrophytes and algae to compete for nutrients at the low concentrations in lakes. These procedures involved (1) competition between several organisms in the same culture for a growth-limiting amount of a nutrient, (2) nutrient replacement in cultures to establish the borderline concentration at which an organism failed to make maximum growth even though the total nutrient supply was adequate, and (3) measurement of rates of nutrient uptake and calculation of Vmax and Km values. The competitive and uptake capacities of various aquatic plants for a specific element differed markedly.

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ABSTRACT

Plant analysis was compared with other frequently used techniques in assays for available nutrients and growth-limiting nutrients in several northern Wisconsin lakes. Data from the different procedures were in poor agreement. The plant analysis bioassay suggested that elements other than P limited plant growth in some of the lakes studied.

As a further development of plant analysis, critical concentrations of a number of elements were established in various macrophytes and algae. The data showed that the critical concentrations for an element can vary greatly in different organisms, so much so that specific critical concentrations must be established for each aquatic species utilized in plant analysis bioassays.

The plant analysis bioassay indicated that K supply, rather than N or P, became limiting for the growth of the macrophytes Myriophyllum spicatum and Ceratophyllum demersum in a shallow eutrophic lake.

Three procedures were developed and tested for evaluating the capacities of macrophytes and algae to compete for nutrients at the low concentrations characteristic of lakes. These procedures involved (1) competition among several organisms in the same culture for a growth-limiting amount of a nutrient, (2) nutrient replacement in cultures to establish the borderline concentration at which an organism failed to make maximum growth even though the total nutrient supply was adequate, and (3) measurement of rates of nutrient uptake and calculation of Vmax and Km values in terms of Michaelis-Menten kinetics. The competitive and uptake capacities of various aquatic plants for a specific element differed markedly.

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SECTION I

CONCLUSIONS

There were two primary aspects to the studies reported. One was concerned with further refinement and testing of plant analysis as a bioassay of nutrient supplies in lakes and streams. The second involved the development and testing of procedures for evaluating relative capacities of aquatic plants to compete for and absorb nutrients at the low concentrations characteristic of lakes and streams.

The primary conclusions can be summarized as follows:

1. Plant analysis and other commonly used assay procedures do not provide results that agree when employed in evaluating nutrient supplies and growth-limiting nutrients in lakes. Even assays by the Provisional Algal Assay Procedure and chemical analysis on aliquots from the same water sample do not agree. The plant analysis bioassay seems to offer several advantages over the other procedures tested.

2. The critical concentrations for a number of elements are now established in a variety of aquatic plants, including macrophytes and algae. In addition to providing essential standard values for bioassays by plant analysis, the critical concentrations indicate interesting variations in the nutritional requirements of the organisms. The data also show that the critical concentration for an element can vary considerably among different organisms, so much so that the same critical values cannot be generally applied even within one taxonomic group. Specific critical concentrations should be established for each species of interest.

3. During the summer months, K supply, as indicated by plant analysis, becomes growth-limiting for the macrophytes Myriophyllum spicatum and Ceratophyllum demersum in a eutrophic southern Wisconsin lake;

N and P supplies remain adequate. The need for caution in generalizing that P is a primary growth-limiting nutrient in all aquatic ecosystems is indicated.

4. The macrophyte Elodea occidentalis and the filamentous green alga Draparnaldia plumosa differ markedly in their capacities to compete for specific nutrients. For example, Elodea is much more effective than Draparnaldia in obtaining K; in contrast, Elodea is very ineffective in competing for Ca at low concentrations.

5. At N and P concentrations approximating critical water concentrations for nuisance algae blooms, Elodea is unable to produce maximum yield even though adequate total amounts of the elements are available. For example, Elodea yield was much reduced at a maintained concentration of 0.30 ppm NO_3-N and 0.02-0.03 ppm P.

6. Vmax and Km values for P uptake, calculated from rates of uptake at various external concentrations, differ considerably among algae and macrophytes. Km values are similar for roots and shoots of Elodea occidentalis. Two mechanisms or carriers for P uptake, which are effective at different external concentrations, seem to function in P uptake by both algae and macrophytes.

The results with the last three techniques demonstrated their potential for obtaining data to explain nuisance aquatic plant distribution in relation to lake fertility and pollution.

SECTION II

RECOMMENDATIONS

The development of plans for the control of nuisance plant growths in lakes and streams optimally requires (1) knowledge of the qualitative and quantitative nutritional requirements of the organisms involved, (2) recognition of unusual nutritional requirements of specific organisms, (3) knowledge of the capacities of the organisms to compete in obtaining essential growth requirements from the environment, and (4) the availability of procedures for accurate evaluation of nutrient supplies in natural waters. This project has contributed to several of these requirements. The primary recommendations on the use and further development of the information obtained are:

1. Plant analysis is a reliable bioassay and should be more widely used in evaluating nutrient supplies and growth-limiting nutrients in aquatic environments.

2. Plant analysis should be further developed and refined as an assay procedure. This should include establishing critical concentrations of various elements in additional nuisance aquatic plants and comparisons of plant analysis with other assay techniques.

3. The possibility that inorganic nutrients other than N and P can be critical in nuisance plant growth should be more widely recognized and tested for.

4. The techniques developed on this project for comparing aggressiveness and competitiveness in nutrient uptake at lake concentrations should be applied to additional organisms, particularly major nuisance aquatic plants. The data obtained should contribute to explanations of the distribution of these plants in relation to lake fertility and pollution.

5. For evaluating competitiveness in nutrient uptake, the two procedures from this project which reflect uptake that results in plant

growth are recommended rather than the procedure which measures only rate of uptake.

SECTION III

INTRODUCTION

The nutritional requirements of most autotrophic plants, including fresh-water algae and macrophytes, are relatively simple. In the absence of toxic conditions, green plants make normal growth upon exposure to suitable temperature, adequate light, water, and inorganic nutrients. External supplies of vitamins, amino acids, and an organic energy source need not be provided. The inorganic nutrients, therefore, become of critical importance in the control of nuisance plants in aquatic environments and in understanding their distribution in various bodies of water.

This project has been concerned primarily with two aspects of the nutritional ecology of nuisance aquatic plants. One aspect was the further development of plant analysis as a reliable bioassay for nutrient supplies and for growth-limiting nutrients in aquatic environments. The other dealt with the development of techniques and the accumulation of data for evaluating the importance of inorganic nutrients in the occurrence of nuisance plants in specific lakes and streams. Both of these aspects relate to possible control of nuisance plants in aquatic ecosystems. For clarity in presentation, each aspect of these studies will be presented in a separate section of this report.

SECTION IV

CRITICAL CONCENTRATIONS OF ESSENTIAL ELEMENTS IN VARIOUS AQUATIC PLANTS

Plant analysis is a technique for evaluating inorganic nutrient availability and growth-limiting nutrients in the environment from the concentrations of those nutrients in plants. The key point in applying the plant analysis technique is to establish the critical concentration of each element of interest in the species under study. The critical concentration, usually established in laboratory experiments, is the minimum concentration of an element, or more often slightly less than the minimum concentration, in a plant which will permit maximum plant yield and growth. Often critical concentrations are determined in index segments rather than entire plants. Nutrient concentrations in index segments more accurately reflect environmental availability of an element than do nutrient concentrations in the entire plant.

If the concentration of an element in a field sample of an aquatic plant, or in the index segment, is above the established critical concentration, the plant was adequately supplied with the element in the environment from which it was collected. If the concentration is below the critical level, supply of that element had become limiting for optimum plant growth.

Development and application of the plant analysis technique in assaying nutrient supplies in aquatic environments has been a primary interest of the Principal Investigator for a number of years. Examples of the use of plant analysis and discussions of possible advantages of that technique over other assay procedures, such as chemical analysis of water samples, have been presented by Gerloff and Krombholz (1966); Gerloff (1969, 1973); and Gerloff and Fishbeck (1973). A number of critical concentrations were established in algae and macrophytes on this project and will be reported. Because of possible use in pollution control efforts, it also seemed desirable to summarize all the

data on critical concentrations in algae and macrophytes obtained by the Principal Investigator over a period of several years. Some of the data were reported previously.

RESULTS

Table 1 includes data on the critical concentrations for various essential elements and the range of concentrations of those elements in index segments of several aquatic angiosperms. Index segments were the first or second one-inch segments cut from the main shoot and laterals. The data on Myriophyllum spicatum, a serious nuisance in Wisconsin lakes, have not been reported previously.

Undoubtedly data on N and P critical concentrations are of most general interest because of their suspected critical roles in eutrophication and promotion of nuisance plant growths. For this reason, critical concentrations of N and P were established for all three macrophytes studied. It would have been highly desirable to determine the critical concentrations for all of the additional essential elements in each species. Unfortunately, these experiments require much time and effort and have not been possible.

The data in Table 2 are for Lemna minor (duckweed). It is an aquatic angiosperm but was not included in Table 1 because with this small organism entire plants rather than index segments were analyzed.

Table 3 presents critical concentrations in several green and blue-green algae. It should be noted that in the original presentation of the Microcystis aeruginosa data it was recognized that the mucilaginous sheath surrounding cells of this organism could complicate use of plant analysis with this species (Gerloff and Skoog, 1957). A procedure was developed for adjusting the critical concentrations to account for variations in the amount of sheath associated with Microcystis obtained from various sources.

One of the most interesting features of the collection and chemical analysis of macrophyte samples from relatively infertile northern Wisconsin lakes in an earlier aspect of this project was their very

Table 1. CRITICAL CONCENTRATIONS AND THE RANGES IN CONCENTRATIONS OF VARIOUS ESSENTIAL ELEMENTS IN SEVERAL AQUATIC ANGIOSPERMS

Element	Index segment	*Elodea occidentalis*		*Ceratophyllum demersum*		*Myriophyllum spicatum*	
		Critical conc.	Range in conc.	Critical conc.	Range in conc.	Critical conc.	Range in conc.
N	2nd 1"	1.60%	1.14-4.32%	1.30%	1.00-2.42%	0.75%	0.61-1.58%
P	2nd 1"	0.14	0.06-0.35	0.10	0.09-0.41	0.07	0.04-0.18
S	2nd 1"	0.08	0.06-0.46	—	—	—	—
Ca	1st 1"	0.28	0.17-0.62	0.22	0.11-0.47	—	—
Mg	2nd 1"	0.10	0.06-0.19	0.18	0.11-0.40	—	—
K	2nd 1"	0.80	0.25-2.51	1.70	1.58-5.10	0.35	0.30-1.13
Fe	1st 1"	60 ppm	40-219 ppm	—	—	—	—
Mn	1st 1"	4.0	2.2-16.7	—	—	—	—
Zn	2nd 1"	8.0	3.6-34.4	—	—	1.8 ppm	1.6-6.9 ppm
Mo	1st 1"	0.15	0.04-6.4	—	—	—	—
B	1st 1"	1.3	0.3-11.2	5.0 ppm	2.3-11.9 ppm	—	—

Table 2. CRITICAL N AND P CONCENTRATIONS AND THE RANGES IN N AND P CONCENTRATIONS IN *Lemna minor* (DUCKWEED)

Element	Critical conc. (%)	Range in conc. (%)
N	0.90	0.40-3.32
P	0.08	0.03-0.73

Table 3. CRITICAL CONCENTRATIONS OF VARIOUS ESSENTIAL ELEMENTS IN SEVERAL GREEN AND BLUE-GREEN ALGAE

Species	%				
	N	P	Ca	Mg	K
Chlorella pyrenoidosa[a]	-	-	0.00	0.15	0.40
Scenedesmus quadricanda[a]	-	-	0.06	0.05	0.25
Draparnaldia plumosa[b]	2.30	0.18	0.03	0.20	2.40
Stigeoclonium tenue[b]	-	-	0.03	0.25	1.90
Microcystis aeruginosa[c]	4.0	0.12	0.04	0.30	0.50
Nostoc muscorum[c]	-	-	0.02	0.25	0.80

[a] unicellular green algae

[b] filamentous green algae

[c] blue-green algae

low Cu content. Based on comparisons with critical concentrations in plants in which Cu nutrition has been studied, Cu concentrations in the macrophytes were low enough to suggest Cu was at times a growth-limiting nutrient in the lakes sampled (Gerloff, 1973).

Much effort has been directed to studying the Cu nutrition and establishing the Cu critical concentration in several macrophytes, particularly Elodea occidentalis. This work required elaborate purification of the nutrient culture media and the environment by standard techniques employed in trace element research (Hewitt, 1966). The very small amounts of Cu present as contamination in average environments can provide sufficient Cu to meet the needs of plants.

Data from experiments on the Cu requirements of several organisms are presented in Table 4. Tomato was included in the experiments because yields with this species for which a Cu requirement is well established served as a bioassay for the effectiveness of the Cu purification procedures. The technique employed was obviously adequate, as indicated by an average yield in triplicate tomato cultures of only 0.65 g. These plants showed symptoms of extreme Cu deficiency. The purified nutrient salts, double-distilled water, and general technique used in the tomato cultures were employed in preparing deficiency cultures of Elodea occidentalis, Myriophyllum spicatum, and the green alga Draparnaldia plumosa. A number of experiments with Elodea were negative, that is Cu deficiency was not established. However, in the experiment reported average yields in control cultures were 52% more than in minus Cu cultures. Copper deficiency definitely was established. The very low Cu requirement of Elodea is indicated by the much greater yield of Elodea (2.99 g) over tomato (0.65 g) when grown in the same volume (2-liters) of nutrient solution. Copper deficiency was not established in Myriophyllum and Draparnaldia even when yields per unit of culture medium were greater than with Elodea. The average growth of Myriophyllum in minus Cu cultures was 8.04 g and in Elodea cultures only 2.99 g. These results suggest Myriophyllum and Draparnaldia have even lower Cu requirements than Elodea, or, although it seems very

Table 4. GROWTH RESPONSE OF SEVERAL PLANT SPECIES WHEN CULTURED IN NUTRIENT MEDIA AND ENVIRONMENTS DEFICIENT IN COPPER

Treatment	Organism	Ave. oven-dry yield, g/21[a]	Ave. Cu conc. in plants, ppm
-Cu	Elodea occidentalis	2.99	0.59
+Cu		4.43	1.47
-Cu	Myriophyllum spicatum	8.04	—
+Cu		7.72	—
-Cu	Draparnaldia plumosa	3.40	—
+Cu		3.15	—
-Cu	Tomato	0.65	—
+Cu		4.80	—

[a] oven-dry weight yields for Elodea, Myriophyllum, and Draparnaldia are the averages of 5 replicates in each treatment; tomato weights are the averages of 3 replicates.

unlikely, that Cu is not required by these plants.

Analyses for Cu in the deficient plants have been difficult because of the extremely low concentrations. Some data on Elodea obtained with a solvent-extraction Atomic Absorption procedure are presented. The Cu concentration in the deficient Elodea was consistently less than a ppm and averaged only 0.59 ppm. This is far below the critical Cu concentration in agricultural crop plants which usually is within the range of 3 to 8 ppm (Chapman, 1966).

The conclusion from the results presented is that the very low Cu concentrations in macrophytes from northern Wisconsin lakes probably do not reflect deficiency and a growth-limiting role of that element, but rather very low requirements for Cu. The physiological basis for this unusually low Cu requirement is of considerable interest. The low Cu requirement also may be a factor in adaptation of these plants to low Cu environments in northern Wisconsin lakes.

DISCUSSION

An obvious question about the application of the plant analysis technique is whether the same critical concentrations are applicable to all species in one taxonomic group, for example among aquatic macrophytes or green algae. It was suggested that the same N and P critical concentrations could be generally used among macrophytes (Gerloff, 1969). However, the data on macrophytes in Table 1 indicate that this is not true. The critical concentrations for N and P are slightly lower in Ceratophyllum demersum than in Elodea occidentalis and are much lower in Myriophyllum spicatum, approximately 50% of the values in Elodea. Values for K also varied widely among the macrophytes, from 0.35% in Myriophyllum to 1.70% in Ceratophyllum. These results establish that, as with agricultural and horticultural species, the use of plant analysis to assay nutrient supplies to aquatic plants requires that critical concentrations must be established for each species of interest.

The primary value of summarizing critical concentrations in Tables 1,

2 and 3 is in their application to nutrient assay by the plant analysis procedure. Unusually high or low critical concentrations also are of interest in indicating high or low nutrient requirements of specific organisms that may be of ecological importance. The critical concentration of N in the blue-green alga Microcystis aeruginosa was 4.0%. This value was nearly twice the second highest critical concentration of 2.30% in the green alga Draparnaldia plumosa. The critical P concentration in Microcystis was 0.12%, a value close to or even below the critical concentrations in most green algae and macrophytes. Relatively large quantities of N must be available for Microcystis growth in lakes and streams. The low Ca critical concentrations in algae, the very low Cu requirements of macrophytes, and the wide variations in K critical concentrations also are of interest. The range in K values suggests this element may play a more significant role in aquatic plant distribution than is presently recognized.

The most consistent duplication in critical concentrations in various experiments was obtained with Lemna minor. Lemna also is relatively easy to grow in synthetic culture media and to quantitatively remove from solutions at harvest. When collected in the field, Lemna should be easier to free of contaminating organisms and debris than would algae. In addition, Lemna provides more uniform tissue than do macrophytes such as Elodea. These features suggest Lemna might be a suitable organism to confine in porous baskets in lakes and to sample periodically for nutrient evaluation by plant analysis (Gerloff, 1973).

SECTION V

COMPARISONS OF PROCEDURES FOR ASSAYING NUTRIENT AVAILABILITY IN AQUATIC ENVIRONMENTS

The availability of assay procedures which correctly measure available nutrients and growth-limiting nutrients for aquatic plants is important in reducing nuisance conditions resulting from heavy growths of these plants. Chemical analysis of water samples probably has been the most used nutrient assay. As an aid in interpreting chemical analyses, critical concentrations of soluble N and P for nuisance bloom development have been proposed, for example by Sawyer (1947) and Vollenweider (1968). These values have been widely used in pollution control efforts. However, as indicated in a review of nutrient assay procedures (Gerloff, 1969), there are problems in obtaining representative water samples for chemical analysis and in evaluating the results.

As an alternative to water analysis, various bioassay procedures have been developed. These include nutrient enrichment cultures, ranging in scale from laboratory flask cultures to field experiments involving large polyethylene bags containing hundreds of liters of water (Schelske and Stoermer, 1971; Powers et al., 1972) and to studies with entire lakes (Schindler, 1974). The technique of plant or tissue analysis uses variations in the concentrations of elements in plants to reflect availability of the elements in the environments in which the plants grow. Fitzgerald (1969) developed bioassays which evaluate environmental P supplies in terms of P extracted from plants during one hour of boiling in water and of N by the rate of NH_4-N uptake in the dark.

As a step in the development of plant analysis as an assay of nutrient supplies for nuisance macrophyte growth, it seemed highly desirable to compare plant analysis with other assays by evaluating available nutrients in a series of lakes. The procedures selected for comparison

were: (1) the Provisional Algal Assay Procedure (PAAP Test), (2) the Fitzgerald bioassay for available P and N, (3) chemical analyses of water samples, (4) plant analysis based on samples from natural populations in the lakes, and (5) plant analysis based on samples from a bioassay macrophyte introduced into the lakes. Data will be presented and discussed in relation to each assay.

EXPERIMENTAL PROCEDURES AND RESULTS

Six northern Wisconsin lakes were selected for sampling. Previous tests (Gerloff and Fishbeck, 1973) showed these lakes varied considerably in general fertility and that some of the lakes were characterized by deficiencies of specific elements. Samples for the assays were collected during the summer of 1973.

Provisional Algal Assay Procedure

In Table 5 average values are presented from the PAAP test on samples of water collected from six lakes during two periods in July and August when aquatic plant growth and demands for nutrients were at a maximum. The green alga Selenastrum capricornutum was the assay organism.

A primary goal of this study was to compare the elements which would be indicated as principal growth-limiting nutrients in lakes sampled by various procedures. In all six lakes the PAAP test showed P to be in least abundant supply for the growth of Selenastrum. There was sufficient P in the lake water samples for an average of 1.66% of maximum growth (range of 1.09 to 2.31%), that is, 1.66% of growth with N, P, Fe and Cu added to the water. There was sufficient N in the water for an average of 29.6% of maximum growth (range of 14.6 to 46.6%).

Unexpectedly, yields actually were less when all essential elements were provided than when only N, P, Fe and Cu were added. Additions of all the elements apparently resulted in heavy metal toxicity or an unfavorable pH or nutrient balance.

Table 5. BIOASSAY FOR GROWTH-LIMITING NUTRIENTS IN SIX NORTHERN WISCONSIN LAKES BY THE PROVISIONAL ALGAL ASSAY PROCEDURE

Lake sampled	Algae growth as % of maximum (N, P, Fe, Cu) with addition of					
	None	(-N) P,Fe	(-P) N,Fe	(-Cu) N,P,Fe	N,P,Fe,Cu	All essential
Little John	1.14	14.9	1.63	103.8	100.0	52.4
Clear	1.00	14.6	1.09	85.1	100.0	51.7
Whitney	1.08	43.1	1.75	55.9	100.0	59.3
Allequash	2.20	46.6	2.31	94.4	100.0	49.3
Salsich	1.02	38.2	1.79	69.1	100.0	85.8
Erickson	0.69	20.0	1.38	60.5	100.0	50.3

Most values are averages from analyses on water samples collected during two sampling periods, July 20 and 21, and August 15 and 16, 1973.

Triplicate samples were run on each treatment of each water sample from a sampling date.

The average dry weight yields of Selenastrum in the cultures represented by a relative growth of 100.0 (N, P, Fe, and Cu added) were 32.5 mg/l for Little John Lake, 35.0 for Clear Lake, 29.7 for Whitney, 26.8 for Allequash, 24.6 for Salsich, and 36.2 for Erickson.

Chemical Analyses of Water Samples

The data in Table 6 are from chemical analyses for several N and P fractions in aliquots of the lake samples which also were analyzed by the PAAP test. The samples were analyzed by the Water Chemistry Laboratory of the Department of Natural Resources at Delafield, Wisconsin. Following collection and prior to analysis, the samples were preserved by adding mercuric chloride as directed by the Water Chemistry Laboratory. Because some analyses were reported as below the limit of detection, data could not be averaged and are presented for both the July and August sampling dates.

The PO_4-P, NO_3-N, and NH_3-N data are of interest in relation to indications that N or P supplies were limiting plant growth. Concentrations of 0.015 ppm inorganic P and 0.30 ppm inorganic N were suggested as critical water concentrations above which nuisance algae blooms could be expected (Sawyer, 1948). These were values for early summer prior to heavy demands on nutrients. The data in Table 6 are from later in the summer. Nevertheless, it is of interest that in all lakes PO_4-P values in the July samples were above the 0.015 ppm Sawyer critical concentration. In the August samples, PO_4-P concentrations in four lakes were below 0.015 ppm. In contrast, values for NO_3-N were below 0.30 ppm in every sample at both sampling dates. The average NO_3-N concentration in the July samples was only 0.05 ppm. All NH_3-N concentrations were reported as less than 0.03 ppm so seem insignificant.

When the ratios of NO_3-N:PO_4-P in the lake water samples are compared with the 12:1 ratio of the critical plant tissue concentrations for N and P in Elodea (1.60% N and 0.14% P), it appears that N is more limiting for growth than P. The average NO_3-N:PO_4-P ratio in the July water samples was 1.4:1 and in the August samples 9.6:1.

Table 6. CHEMICAL ANALYSES FOR N AND P FRACTIONS IN WATER SAMPLES FROM SIX NORTHERN WISCONSIN LAKES

Lake sampled	Concentration (ppm)												Ave. alk.	Ave. pH
	PO_4-P		Total P		NO_3-N		NH_3-N		NO_2-N		Total N			
	I	II	I	II	I	II	I	II	I	II	I	II		
Little John	0.043	0.016	0.08	0.03	0.04	0.25	<0.03	<0.03	0.006	0.002	0.91	0.83	46	7.6
Clear	0.023	0.009	0.05	0.03	0.07	0.11	<0.03	<0.03	0.002	0.002	0.84	0.64	33	7.3
Whitney	0.016	0.013	0.07	0.01	0.04	0.11	<0.03	<0.03	<0.002	<0.002	1.03	0.81	20	7.2
Allequash	0.037	0.017	0.08	0.03	0.08	0.04	<0.03	<0.03	0.002	0.002	1.13	0.71	34	7.5
Salsich	0.067	0.009	0.04	0.01	<0.04	0.09	<0.03	<0.03	0.002	0.000	0.85	0.57	12	7.0
Erickson	0.038	0.005	0.04	0.02	0.05	0.06	<0.03	0.04	0.002	<0.002	0.97	0.65	23	7.2

I, from July 20-21 sampling period; II, from August 15-16 sampling.

Fitzgerald Tests for Available N and P

The data in Table 7 are the results of bioassays for available N and P in lake water samples by the Fitzgerald tests. One problem in using the Fitzgerald assays is the lack of definite standards against which to evaluate analyses of particular species collected from lakes. In a publication describing the procedures (Fitzgerald, 1969), it is suggested that, in general, uptake rates in excess of 15 µg NH_4-N/10 mg dry tissue/hr indicate N deficient plants. Extractable PO_4-P values of 190 to 200 µg/100 mg dry tissue indicate plants adequately supplied with P; values of 50 to 70 µg/100 mg suggest P deficient plants. A laboratory calibration trial with Elodea on this project indicated the 50 to 70 µg/100 mg P values were slightly high for that organism. Borderline values for Elodea of 25 to 30 µg/100 mg seemed more suitable.

Table 7. BIOASSAYS BY THE FITZGERALD TESTS FOR P (HOT WATER EXTRACTABLE) AND N (NH_4-N UPTAKE IN THE DARK) IN Elodea occidentalis COLLECTED FROM NORTHERN WISCONSIN LAKES

Lake sampled	P extracted (µg/100 mg dry Elodea)	NH_4-N uptake (µg/hr/10 mg dry Elodea)
Little John	186	None
Clear	146	None
Whitney	94	None
Allequash	293	None
Salsich	193	None
Erickson	176	None

Values presented are averages for samples collected during two periods, July 20-22, 1973, and August 15-19, 1973.

There was no uptake of NH_4-N by any Elodea sample. Thus, Elodea from every lake was indicated to be adequately supplied with N. Also every extractable PO_4-P value was above the 50 to 70 µg/100 mg value indicative of P deficiency. Most values were close to or above the 190 to 200 µg/100 mg dry tissue associated with adequate P. The average value was 181. The PO_4-P extraction tests did indicate that Whitney Lake contained much less available P than the other lakes. The average extractable P value for Whitney was 94, only 47% of the average for the other five lakes.

Nutrient Bioassay by Plant Analysis

The data in Table 8 are the results of total N and P analyses of second one-inch index segments of Elodea collected from the various lakes during July and August. These analyses were evaluated for indications that N or P had become growth-limiting by comparisons with 1.60% N and 0.14% P established critical concentrations (Gerloff and Fishbeck, 1973).

No sample was below the critical N concentration of 1.60%. However, the August Elodea sample from Clear Lake was close to the critical concentration indicating borderline N deficiency. In contrast, the average P concentration in Elodea from Clear Lake was equal to the highest value from any lake. The analyses of samples from Whitney showed that P had become limiting for Elodea growth in that body of water. Phosphorus concentrations in samples from both dates were below the 0.14% critical concentration. Average P concentrations in samples from all other lakes were approximately double the critical concentration and varied only within the range of 0.25 to 0.27%.

Analyses for the concentrations of major element cations in appropriate index segments are presented in Table 9. In earlier work, the critical concentration for Ca in Elodea was established at 0.28%; for Mg, 0.10%; and for K, 0.80%.

Potassium concentrations in the Elodea averaged 2.33% with the lowest value at 1.70%. All K concentrations were at least double the

Table 8. TOTAL N AND P CONCENTRATIONS IN SECOND ONE-INCH INDEX SEGMENTS OF *Elodea occidentalis* COLLECTED FROM NORTHERN WISCONSIN LAKES

Lake sampled	Total N (% dry wt.)			Total P (% dry wt.)		
	I	II	Ave.	I	II	Ave.
Little John	1.78	2.11	1.95	0.23	0.27	0.25
Clear	1.96	1.65	1.81	0.31	0.23	0.27
Whitney	2.18	2.15	2.17	0.11	0.14	0.13
Allequash	2.16	2.34	2.25	0.20	0.33	0.27
Salsich	3.02	3.57	3.30	0.21	0.29	0.25
Erickson	3.10	3.16	3.13	0.21	0.30	0.26

I, samples collected July 20-21; II, samples collected August 15-16.

Table 9. MAJOR ELEMENT CATION CONCENTRATIONS IN INDEX SEGMENTS OF *Elodea occidentalis* COLLECTED FROM NORTHERN WISCONSIN LAKES

Lake sampled	Ca conc. (%)			Mg conc. (%)			K conc. (%)		
	I	II	Ave.	I	II	Ave.	I	II	Ave.
Little John	1.25	--	1.25	0.10	0.12	0.11	2.73	1.70	2.22
Clear	0.67	0.83	0.75	0.11	0.11	0.11	2.38	1.75	2.07
Whitney	0.68	0.62	0.65	0.11	0.12	0.12	1.89	2.21	2.05
Allequash	0.73	0.86	0.80	0.11	0.13	0.12	2.01	2.17	2.09
Salsich	0.20	0.31	0.26	0.11	0.12	0.12	2.44	3.24	2.34
Erickson	0.29	0.36	0.33	0.11	0.13	0.12	2.31	3.08	2.70

The index segment for Ca was the first one-inch of the main stems and laterals; for K and Mg, the second one-inch was used.

critical concentration, so there was no indication that this element became growth-limiting. In contrast, Mg concentrations were only slightly above the critical value in all six lakes and Ca concentrations were close to or below the critical concentration in two lakes (Salsich and Erickson). The Ca concentrations in the Elodea correlate well with the alkalinity values for the lakes reported in Table 6. The highest Ca concentration (1.25%) was in plants from Little John Lake which also had the hardest water (alkalinity of 46); the lowest Ca concentration (0.20%) was from Salsich which had the softest water (alkalinity of 12). The Ca concentration in the Lake Whitney samples was relatively high even though the alkalinity was only 20. This probably is because P was the limiting element in that lake.

Data from analyses of the Elodea samples for Fe, Mn, Cu, B, and Zn are presented in Table 10. All values are above established critical concentrations indicating that none of these elements was limiting Elodea growth. In comparison with critical Cu concentrations in terrestrial species (4-7 ppm) some of the Cu values for Elodea are very low. In earlier work similar low Cu concentrations were interpreted as indicating Cu deficiency in Elodea. Additional studies have shown Elodea has an extremely low Cu requirement in comparison with other angiosperm plants. Therefore, it is doubtful the low concentrations in the Elodea indicate a growth-limiting role of that element. Copper might be critical, however, for other aquatic plants.

Plant Analysis of Introduced Elodea

A second application of plant analysis in this study was to introduce Elodea into the lakes in such a way that it could be routinely sampled and analyzed to indicate nutrient supplies. The Elodea was floated near the water surface in plastic-mesh, porous cylinders as described in earlier work (Gerloff, 1973). At the surface, Elodea would directly derive nutrients only from the water and not from both the water and the bottom muds. It was anticipated that this would result in a more suitable general assay for nutrients in lake water than would sampling macrophytes rooted in bottom muds.

Table 10. TRACE ELEMENT CONCENTRATIONS IN INDEX SEGMENTS OF Elodea occidentalis COLLECTED FROM NORTHERN WISCONSIN LAKES DURING JULY AND AUGUST

Lake sampled	Concentration (ppm)														
	Fe			Mn			Cu			B			Zn		
	I	II	Ave.	I	II	Ave.	I	II	Ave.	I	II	Ave.	I	II	Ave.
Little John	1020	--	1020	378	--	378	2.5	--	2.5	10.0	--	10.0	21	--	21
Clear	376	461	419	>800	845	--	3.7	6.3	5.0	9.3	15.5	12.4	40	30	35
Whitney	280	161	221	108	79	94	2.6	4.3	3.5	9.5	10.3	9.9	54	46	50
Allequash	763	356	560	457	287	372	1.9	7.8	4.9	12.4	14.2	13.3	45	80	63
Salsich	499	383	441	37	46	42	2.6	5.1	3.9	9.8	14.9	12.4	64	52	58
Erickson	442	568	505	72	94	83	9.5	5.4	7.5	12.3	16.7	14.5	114	47	81

The index segment for Fe, Mn, and B was the first one-inch of main stems and laterals; for Cu and Zn, the second one-inch.

The critical concentrations are 60.0 ppm for Fe, 4.0 for Mn, 0.8 for Cu, 1.3 for B, and 8.0 ppm for Zn.

Results with the assay baskets were disappointing. Neither Elodea brought from the laboratory as inoculum nor Elodea picked from local populations grew well enough in the baskets to justify presenting the limited analytical data obtained. Elodea from lake populations seemed to grow somewhat better than laboratory-cultured plants. Problems with this technique must be solved before it can be considered a successful application of the plant analysis bioassay.

DISCUSSION

The point of primary interest in this study is the degree of agreement in predictions of growth-limiting nutrients by the various procedures. On this point, two general observations seem justified. First, there was satisfactory agreement among the three bioassays in evaluating N and P supplies. All three procedures indicated P was more likely to become a growth-limiting nutrient in the lakes than was N. This was true even though the PAAP test is based on water samples only while the Fitzgerald tests and plant analysis involve Elodea which can absorb nutrients both from water and mud substrates. The Fitzgerald tests and plant analysis agreed in indicating that in all the lakes Elodea was adequately supplied with N. The two tests also agreed in indicating that P supply was least adequate for Elodea in Whitney Lake.

A second general point was that the conclusions from the bioassays and chemical analyses of water samples did not agree. Chemical analyses indicated N supplies would more readily become limiting than would P; the bioassays indicated P would first be in the limiting role. This difference could not be due only to the absorption by Elodea nutrients from both water and bottom muds because the PAAP test is a bioassay employing aliquots of the water samples used in chemical analyses. The most obvious explanation is, of course, that the base values against which the chemical analyses were evaluated are not appropriate. Relatively small differences in the base values in any of the tests could drastically modify the conclusions derived from the tests.

The results obtained do not permit a conclusion that one procedure is more reliable than the others. This was not an initial goal of the study. However, as a general observation it can be stated that bio-assays in which plants growing in natural environments are the sampling devices and can reflect all factors which affect nutrient availability over a period of time seem to offer advantages over assays based on water samples taken infrequently. Then the rate of replacement of utilized nutrients is not adequately considered. This replacement may be from organism decay, release from bottom sediments, N fixation, and nutrient inflow. The reliability of the plant analysis assay was supported by agreement of the 1973 data and data from 1971 using the same technique in these lakes. Of the lakes sampled, in both years Whitney Lake was indicated to be most deficient in P; Clear Lake was most deficient in N; and Erickson Lake was indicated as low in Ca.

SECTION VI

POTASSIUM AS A GROWTH-LIMITING NUTRIENT FOR *Myriophyllum spicatum* IN A EUTROPHIC LAKE

Lake Wingra, located within the city limits of Madison, Wisconsin, is a small (140 hectares) and shallow (maximum depth, 6.4 meters) lake with a severe aquatic weed problem. The lake is primarily spring-fed and the southern shore, which is undisturbed woods, is included in the University of Wisconsin Arboretum. The northern shore is dominated by recreational and residential areas (Bauman, Hasler, Koone, and Teraguchi, 1973). The dominant vegetation in the lake is *Myriophyllum spicatum* (relative frequency 64%), a submerged, rooted angiosperm that has been introduced from Europe (Nichols, 1971).

The problem of accelerated lake eutrophication has stimulated the recent proliferation of techniques for assessing the nutrient status of natural waters and of investigations of the movement of mineral elements through aquatic ecosystems. Most of these studies have concentrated on N and P, and in many cases one or the other has been found to be limiting plant growth. Lake Wingra has been the site of one such study as part of the US/IBP Eastern Deciduous Forest Biome Project. One phase of the general project of modeling physical, chemical, and biological processes in the Lake Wingra Basin focused primarily on the movement of P through the ecosystem.

This paper presents data from application of the bioassay known as plant analysis (Gerloff and Krombholz, 1966) in evaluating the availability of three major nutrient elements, N, P, and K, for plant growth in Lake Wingra and in determining if one of these elements became limiting for plant growth. This study was stimulated by the extensive work on Lake Wingra in the IBP program, but was not a part of that project. In addition to the samples of *Myriophyllum spicatum*, samples of *Ceratophyllum demersum*, a less abundant, weakly-

rooted, submerged macrophyte were also collected and analyzed.

EXPERIMENTAL PROCEDURES

Myriophyllum spicatum was isolated and cultured in synthetic media by the general techniques previously described (Gerloff, 1973). The composition of the nutrient solution employed is presented in Table 11. This is a slight modification of a nutrient solution described earlier (Gerloff and Krombholz, 1966). The trace elements are in the concentrations presented in a modified Hoagland's solution for higher plants (Johnson et al., 1957). Iron was provided as a mole:mole complex with EDDHA (ethylene-diamine di-[o-hydroxyphenyl-acetate]). Additional Fe-EDDHA complex was added to the cultures whenever the pink color resulting from the complex disappeared. All nutrient media were autoclaved before use.

To establish N, P, and K critical concentrations in Myriophyllum, the plants were grown for 4 to 6 weeks in a series of triplicated cultures similar in all respects except for the element in question. Each flask was inoculated with two two-inch shoot tips.

The cultures were continued until there was a marked growth difference between those containing the lowest and highest levels of the element varied and also a noticeable difference at the intermediate levels. Upon harvest, the plants of each flask were dissected into three categories: first one-inch segment from the shoot growing tip, second one-inch segment from the growing tip, and remaining plant parts. Roots and any flowers were put in the remainder category. The first one-inch segment was measured by drawing the leaves on the tip forward and measuring from the tips of the leaves. The second one-inch was measured as an inch segment of stem. After harvest, the plants were dried for 48 hours in a forced-draft oven at 60-65°C, weighed, ground in an agate mortar and pestle, and analyzed. Nitrogen analyses were by a semi-micro Kjeldahl procedure; P determinations were by a vanado-molybdate (yellow complex) colorimetric procedure following dry-ashing; and K analyses were by flame emission with a Coleman flame photometer after extraction of the tissue with 1 N ammonium acetate.

Table 11. COMPOSITION OF A MODIFIED GERLOFF AND KROMBHOLZ SOLUTION USED FOR THE CULTURE OF ANGIOSPERM AQUATIC PLANTS

Salt	1.0 M stock solution per 1 final solution (ml)	Element in final solution (ppm)
KNO_3	0.8	N - 33.6
$Ca(NO_3)_2 \cdot 4H_2O$	0.8	K - 39.0
$MgSO_4 \cdot 7H_2O$	0.4	Ca - 32.0
KH_2PO_4	0.2	P - 6.2
		S - 12.8
		Mg - 9.6
KCl[a]		Cl - 1.77
H_3BO_3[a]		B - 0.27
$MnSO_4 \cdot H_2O$[a]		Mn - 0.27
$ZnSO_4 \cdot 7H_2O$[a]		Zn - 0.13
$CuSO_4 \cdot 5H_2O$[a]		Cu - 0.03
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$[a]		Mo - 0.01
Fe•EDDHA		Fe - 0.56

[a]Trace element stock solutions were prepared at 1000x the concentration of the final solution. One ml of each stock solution was added to each liter of the final culture medium.

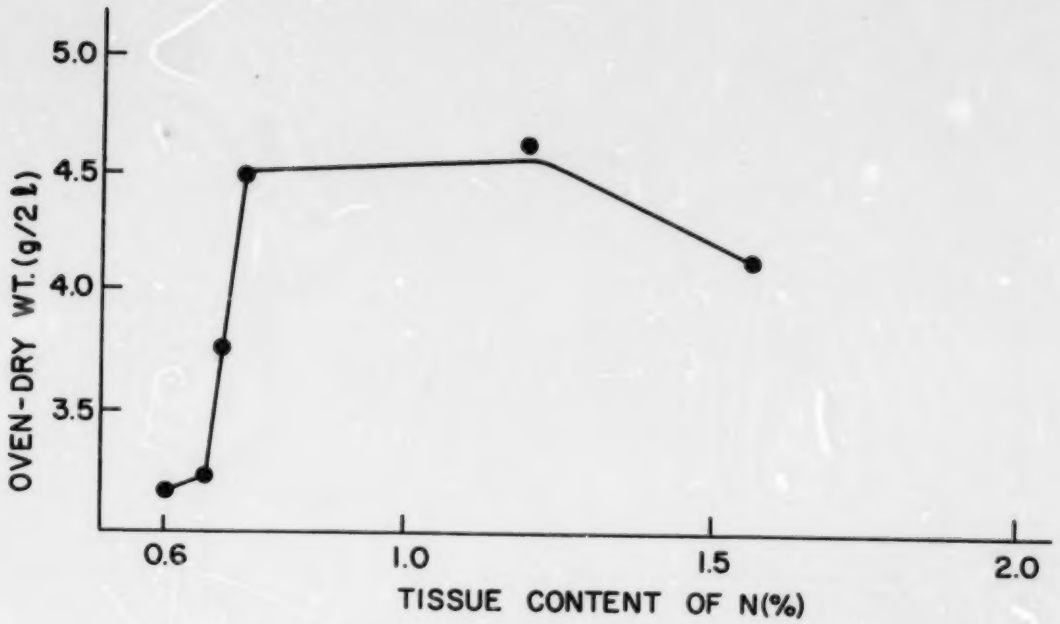

Figure 1. The relationship between yield and total nitrogen content of the second one-inch terminal segments of Myriophyllum spicatum grown in solutions of varying nitrogen content.

RESULTS

Figures 1, 2, and 3 present the curves from which critical concentrations of N, P, and K were established in Myriophyllum spicatum. Because of the mobility of N, P, and K in plants (Gerloff, 1973), second one-inch sections were selected as index segments and critical concentrations were based on analyses of those segments. The data show that concentrations of each element varied over a wide range, N from 0.61 to 1.58%, P from 0.04 to 0.18%, and K from 0.30 to 1.13%. The critical concentrations were established as 0.75% N, 0.07% P, and 0.35% K. These values are the concentrations associated with yields about 5% below the maximum. Element concentration above the

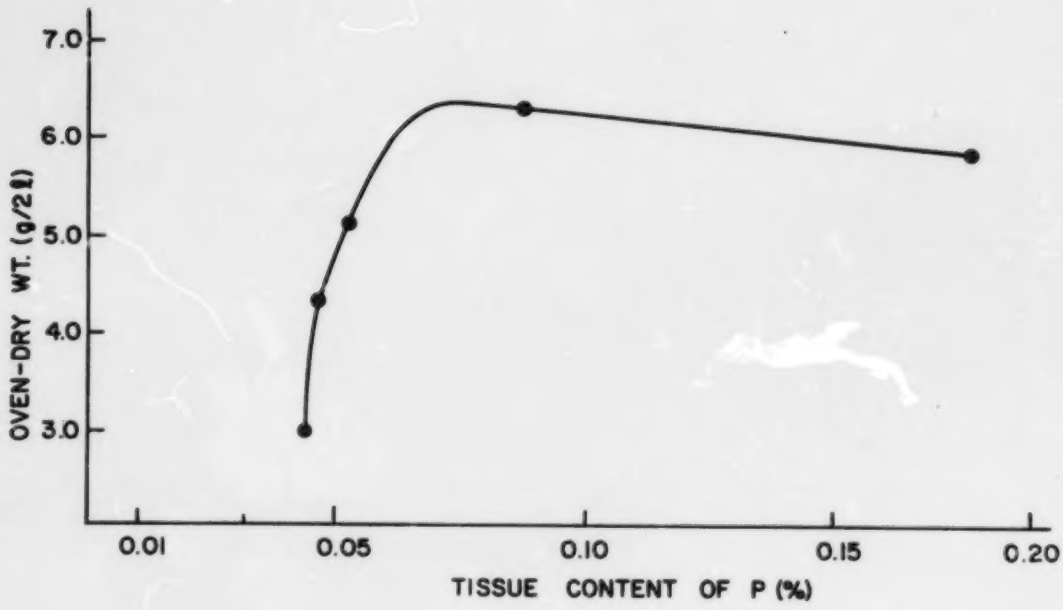

Figure 2. The relationship between yield and total phosphorus content of the second one-inch terminal segments of Myriophyllum spicatum grown in solutions of varying phosphorus content.

critical levels represented luxury consumption that did not produce further yield increases. In fact, the highest plant concentrations of N and P were slightly inhibitory.

Following establishment of N, P, and K critical concentrations in Myriophyllum, nutrient availability in Lake Wingra was evaluated by comparisons of the analyses of index segments collected from two sites in the lake with the critical concentrations. The analytical data are presented in Table 12.

There were no indications that supplies of either N or P became limiting for Myriophyllum growth at any time during the three years

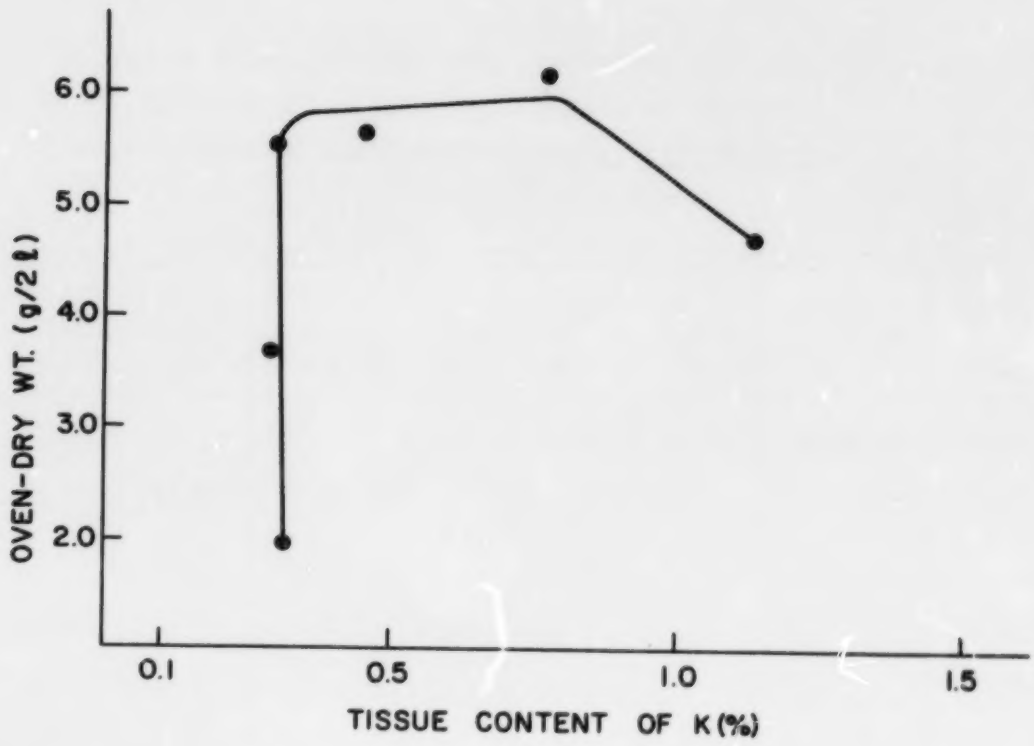

Figure 3. The relationship between yield and total potassium content of the second one-inch terminal segments of Myriophyllum spicatum grown in solutions of varying potassium content.

of sampling. The lowest N concentration in second one-inch segments from Site 1 was 2.72% in the July 3, 1973, sample; the lowest in Site 2 samples was 1.85% in the August 10, 1973, sampling. The average N concentration in samples from both sites was 2.78% or 3.7x the critical concentration.

The lowest P concentration in second one-inch segments from Site 1 was 0.26% and from Site 2, 0.14%. These minimum values are respectively 4x and 2x the 0.07% critical P concentration.

In contrast to N and P there were definite indications that K was a limiting nutrient for Myriophyllum growth in Lake Wingra. At both sites the K concentration in 8 of 9 samples of second one-inch

Table 12. NITROGEN, PHOSPHORUS, AND POTASSIUM CONCENTRATIONS (OVEN-DRY BASIS) IN SECOND ONE-INCH SEGMENTS OF *Myriophyllum spicatum* SHOOTS COLLECTED FROM LAKE WINGRA

Year	Date sampled	Site 1			Site 2		
		% N	% P	% K	% N	% P	% K
1971	July 26	2.81	0.29	0.27	2.52	0.18	0.23
	Aug. 19	2.92	0.29	0.24	1.99	0.14	0.19
1972	June 15	3.53	0.40	0.19	2.30	0.17	0.46
	June 29	3.96	0.45	0.20	2.10	0.16	0.19
	July 27	3.78	0.44	0.27	2.21	0.18	0.26
	Aug. 10	4.30	0.61	0.42	1.85	0.18	0.31
	Aug. 31	3.43	0.33	0.30	2.43	0.25	0.30
1973	July 3	2.72	0.26	0.20	2.23	0.20	0.22
	Aug. 6	3.11	0.44	0.30	1.89	0.21	0.25

segments was below the 0.35% critical concentration. The lowest K concentration at each site was 0.19%; the average K concentration in the 18 samples was only 0.24%. This suggests severe K deficiency.

Analysis of a number of samples of *Ceratophyllum demersum* collected at the same sites as the *Myriophyllum*, are presented in Table 13. The critical concentrations for *Ceratophyllum*, based on second one-inch index segments, are higher than those for *Myriophyllum*. They are 1.30% N, 0.10% P, and 1.70% K (Gerloff, 1973). Comparisons of these values and the analyses of the Lake Wingra samples indicate

Table 13. NITROGEN, PHOSPHORUS, AND POTASSIUM CONCENTRATIONS (OVEN-DRY BASIS) IN SECOND ONE-INCH SEGMENTS OF *Ceratophyllum demersum* SHOOTS COLLECTED FROM LAKE WINGRA

Year	Date sampled	Site 1			Site 2		
		% N	% P	% K	% N	% P	% K
1971	July 26	3.27	0.32	2.49	--	--	--
	Aug. 19	2.00	0.21	2.11	--	--	--
1972	June 15	3.19	0.36	3.57	--	--	--
	June 29	3.71	0.50	2.70	--	--	--
	July 27	3.05	0.29	2.01	--	--	--
	Aug. 10	2.90	0.32	2.45	2.48	0.18	2.55
	Aug. 31	2.55	0.36	3.10	1.88	0.16	1.38
1973	July 3	2.75	0.39	3.27	2.41	0.31	2.80
	Aug. 6	2.51	0.39	2.37	2.02	0.18	2.14

that N and P supplies did not approach limiting levels. The lowest N concentration was 1.88% (August 31, 1972), and the average was 2.67%. The lowest P concentration was 0.16% (August 31, 1972) while the average concentration was 0.30%.

In contrast to the *Myriophyllum* data, the K concentration in *Ceratophyllum* dropped below the 1.70% critical concentration in only one sample. That was to 1.38% on August 31, 1972. The average K concentration was 2.53%, or 1.48x the critical concentration. Even though K was less limiting for *Ceratophyllum* than for *Myriophyllum*, the analyses indicated K would more likely limit *Ceratophyllum* growth than would N or P.

DISCUSSION

This study is unique in indicating that K rather than N or P became limiting for the growth of a nuisance aquatic plant in a eutrophic lake. This probably is the first evidence for K deficiency in plants in natural aquatic environments. The occurrence of K deficiency in Lake Wingra Myriophyllum supports the suggestion (Gerloff, 1968) that it is incorrect to assume that P is the primary growth-limiting nutrient in all lakes. As in terrestrial environments, the principal growth-limiting nutrient in aquatic environments most often will be P or N but in specific environments it may be any other major element or even a trace element.

One procedure for reducing nuisance plant growths in lakes is to lower the input of a particular element, or elements, especially the element considered most likely to limit plant growth. One of the easier elements to control is P. The present study shows that reduced entry of K into Lake Wingra would have a greater effect on the standing crop of Myriophyllum than would reduced P input. This is not to advocate discontinuance of efforts to reduce P input, but to recognize that the results of such an effort may not have the expected effects in reducing Myriophyllum biomass.

Several aspects of this study relate to the practical application of plant analysis as a nutrient bioassay. First, the critical concentrations of N, P, and K were much lower in Myriophyllum spicatum than in other macrophytes in which critical concentrations have been established. Therefore, critical concentrations must be determined for each species of interest. Secondly, the data obtained demonstrated that caution should be used in generalizing that an element is limiting the growth of all species in a lake from below-the-critical-concentration values in one species. The K supply was indicated as severely limiting for growth of Myriophyllum but only on the borderline of limiting for Ceratophyllum. Thirdly, the results show that differences in nutrient availability within a lake can be demonstrated with plant analysis. As shown in Tables

12 and 13, N and P concentrations in index segments were consistently lower at Site 2 than Site 1.

Because use of plant analysis in aquatic environments is relatively new and because of unexpected indications of K deficiency, several critical steps in the plant analysis procedure were repeated. The critical concentrations for N, P, and K were verified in different experiments and the K analyses were checked by the Wisconsin Alumni Research Foundation Laboratories with a Jarrell-Ash Multichannel Spectrometer. It also was considered possible that the second one-inch segments analyzed for K were not as carefully measured as required. However, this would not have resulted in major differences in the results, because the critical concentration in the first one-inch segment was only 0.05% (0.35% vs. 0.40%) greater than the second one-inch. Nevertheless, it would be desirable to verify by other diagnostic techniques the primary conclusion of this study that K supply limited growth of Myriophyllum spicatum in Lake Wingra.

SECTION VII

COMPETITION FOR GROWTH-LIMITING AMOUNTS OF NUTRIENTS MADE AVAILABLE AT VERY LOW CONCENTRATIONS IN MIXED CULTURES OF AQUATIC PLANTS

Nuisance conditions in lakes, even in one general area, are not caused by the same species of algae and macrophytes. Hard water lakes are characterized by different species than are soft water lakes; blue-green algae often become the dominant organisms following eutrophication; and Myriophyllum spicatum frequently is the primary macrophyte after its introduction into Midwestern lakes. Differing responses to nutrient concentrations must be one factor determining aquatic plant distribution. The limited information available on this point has been obtained primarily through attempts to correlate nutrient concentrations in lakes with relative organism abundance.

A primary aspect of this project has been to develop laboratory procedures which would permit comparisons of the capacities of aquatic plants to compete for nutrients that were made available at very low concentrations characteristic of lakes and streams. Tests have been made with three different procedures and techniques. One procedure involved growing two or more aquatic plants in the same culture flask with adequate supplies of all essential elements except one. The organisms were forced to compete for that element by making small additions to the culture at intervals during the growth period. The total amount of the element provided was less than adequate for optimum growth of both organisms.

A second technique involved solution-replacement cultures in which plants were confined to a culture flask in which the nutrient solution containing a specific element at a low concentration was frequently replaced. The total amount of the element made available to the plants was more than required for maximum growth. The goal with this technique was to establish the borderline concentration of the element

under study at which the plants in culture could not absorb enough of the element to produce maximum growth.

A third approach was to establish the rates of uptake of key elements at various external concentrations according to Michaelis-Menten kinetics. Uptake by various species then would be calculated as Km and Vmax values and comparisons of accumulation capacities of the species would be made. This technique perhaps would give different results than the first two because the rate of uptake measured in short-term experiments might not be associated with optimum utilization of an element in plant growth.

Results with these three procedures will be presented in Sections VII, VIII and IX of this report.

EXPERIMENTAL PROCEDURES

The experimental organisms in the mixed cultures were two macrophytes, Elodea occidentalis and Myriophyllum spicatum, and a filamentous green alga, Draparnaldia plumosa. It was essential that the organisms employed could be readily separated at the termination of an experiment when dry-weight yields were determined. It also seemed desirable that the organisms should grow at about the same rate so that one species would not dominate a culture. Because growth of macrophytes is slowly initiated in freshly inoculated cultures, macrophytes were inoculated into mixed cultures 5 days prior to Draparnaldia.

Plants were grown in two-liters of medium in three-liter Florence flasks. The culture medium was the solution described in Table 11 with the trace element concentrations slightly modified. This solution proved a satisfactory medium for both the Draparnaldia and the macrophytes. Other culture conditions were as described earlier (Gerloff and Krombholz, 1966). To insure that the desired low concentrations of an element would not be exceeded, the growth-limiting element in a specific experiment was added to cultures at intervals during the growth period. All treatments were in triplicate.

The total amount of an element made available in deficient cultures was approximately 50% of the amount considered necessary to maintain the critical concentration in the anticipated plant yield. The frequency of addition of the element increased as the growth period progressed. For example, in the N experiment a N addition equivalent to 0.30 ppm in two-liters of solution was made each 24 hours of the first 5 days of the culture period; two additions were made each 24 hours during the last 6 days.

At the termination of an experiment, the plants were carefully separated. Elodea and Myriophyllum were lifted from the culture medium, and adhering algae were carefully washed off to be combined with the algae from the culture medium. Draparnaldia was recovered from the culture solution by filtration through 270-mesh bolting cloth held in a Büchner funnel. After drying at 65-70°C and weighing, the plants were analyzed. Nitrogen determinations were by a semi-micro Kjeldahl procedure, P by a stannous-reduced phosphomolybdate yellow-color procedure on dry-ashed residues, Ca by atomic absorption analysis of dry-ashed tissues, and K by atomic emission following extraction with N NH_4Ac.

RESULTS

The relative capacities of two species to compete for an element in the experiments reported are indicated by (1) the percentage of the total yield represented by each species when grown at a growth-limiting supply of the element and (2) a comparison of the proportion of growth represented by each species under adequate and less than adequate quantities of the element. Actual reduction of the supply of an element to a growth-limiting concentration in an experiment is evident from reduced combined yields of the organisms at the low element concentration and also element concentrations in the harvested plants close to or below recognized critical concentration. It was important that differences in growth rate were not primarily responsible for yield differences of the mixed-culture organisms. Marked differences in growth rates would be apparent in comparisons of yields

of the two organisms when each was grown separately and not competing for a nutrient.

The data in Table 14 indicate relatively little difference in the efficiency of NO_3-N utilization by Elodea and Draparnaldia. At the low concentration of NO_3-N (0.30 ppm), yields were approximately equal (47% vs. 53% for Elodea and Draparnaldia respectively). The change in the proportion of total yield represented by each species at the two NO_3 concentrations suggests somewhat greater efficiency in utilization by Draparnaldia. Yield represented by Draparnaldia, when the organisms were grown together, increased from 36% at 33.6 ppm N to 53% at 0.30 ppm.

When grown alone, Elodea yield decreased markedly at the lower NO_3 concentration (from 1.07 to 0.37 g) while Draparnaldia yield decreased only slightly from 0.49 to 0.41 g. Total yield in the mixed cultures was only about one-half the yield at high NO_3 (0.60 vs. 1.25 g).

The data in Table 15 are from an experiment involving competition for a limited P supply. Again yields were about equal when the two organisms were grown in competition at the 0.075 ppm concentration of P (54% for Elodea and 46% for Draparnaldia). However, comparisons of yields at high and low P suggest a superior competitive capacity of Draparnaldia. The proportion of total yield represented by Elodea decreased from 89 to 54% while the yield represented by Draparnaldia increased from 11 to 46%. When cultured separately, Elodea yield decreased from 1.71 g to 0.81 g at the lower P concentrations while yield of Draparnaldia remained constant at 0.67 g. Total yield of the two organisms grown in combination was 1.48 g at 6.2 ppm P and only 1.03 g at 0.075 ppm. Under low P, the P concentration in each organism was at or close to its respective critical concentration of 0.14% for Elodea and 0.18% for Draparnaldia, thus indicating P deficient plants.

Table 14. GROWTH AND TOTAL N CONTENT OF Elodea occidentalis AND Draparnaldia plumosa WHEN COMPETING FOR A GROWTH-LIMITING SUPPLY OF N IN A MIXED CULTURE

NO_3-N conc. (ppm)	Elodea					Draparnaldia					Mixed culture yield (g)
	Yield (g)		Prop. of yield (%)	N content (%)		Yield (g)		Prop. of yield (%)	N content (%)		
	Alone	+Drap.		Alone	+Drap.	Alone	+Elodea		Alone	+Elodea	
33.6	1.07	0.80	64	4.44	3.85	0.49	0.45	36	6.83	5.07	1.25
0.30	0.37	0.28	47	2.68	1.88	0.41	0.32	53	1.70	1.51	0.60

All yields are average oven-dry weights from triplicate cultures.

Table 15. GROWTH AND P CONTENT OF Elodea occidentalis AND Draparnaldia plumosa WHEN COMPETING FOR A GROWTH-LIMITING SUPPLY OF P IN A MIXED CULTURE

P conc. (ppm)	Elodea					Draparnaldia					Mixed culture yield (g)
	Yield (g)		Prop. of yield (%)	P content (%)		Yield (g)		Prop. of yield (%)	P content (%)		
	Alone	+Drap.		Alone	+Drap.	Alone	+Elodea		Alone	+Elodea	
6.2	1.71	1.32	89	0.72	0.62	0.67	0.16	11	1.30	1.29	1.48
0.075	0.81	0.56	54	0.21	0.12	0.67	0.47	46	0.18	0.16	1.03

In contrast to its effectiveness in N and P uptake, Draparnaldia was very ineffective in competition for a limited K supply (Table 16). At 0.39 ppm K, only 3% of the yield in mixed cultures was represented by the alga. Under an adequate K supply, Draparnaldia did compete quite effectively with Elodea producing 38% of the yield in a mixed culture.

The analytical data also support Draparnaldia's inefficiency in K uptake. The K critical concentration in Elodea has been established as 0.80%; in Draparnaldia, as 2.40%. When grown alone with adequate K, the K concentration in Draparnaldia was 4.23%, well above the critical concentration. However, in competition with Elodea the K concentration was slightly below the critical concentration at 2.02%. Under growth-limiting K, the K concentration in Elodea remained slightly above the critical concentration at 0.97% in competition with Draparnaldia. However, the K concentration in Draparnaldia was far below the critical concentration at only 1.08%.

The data in Table 17 show that the relative efficiency of the two organisms in Ca uptake was the reverse of effectiveness in K uptake. Elodea was completely ineffective in obtaining adequate Ca at 0.8 ppm. In the mixed culture, only 5% of the yield was represented by Elodea. Under adequate Ca, 69% of the yield was Elodea. The very poor performance of Elodea under low Ca seems due to a general inability to absorb Ca at low concentrations. Even when grown alone, there was almost no growth at 0.8 ppm Ca. In contrast, in the previous experiment Draparnaldia grew very well at 0.39 ppm K in the absence of competition from Elodea.

The results on competition for Mg are quite comparable to the N and P data (Table 18). At the growth-limiting supply of Mg (0.2 ppm), yields of the two organisms were about equal (59% and 41% for Elodea and Draparnaldia, respectively). The increase in the proportion of the yield represented by Draparnaldia between the high and low Mg supply (15 to 41%) suggests Draparnaldia is somewhat more competitive. The Mg concentration in Elodea grown in competition for 0.2 ppm Mg

Table 16. GROWTH AND K CONTENT OF *Elodea occidentalis* AND *Draparnaldia plumosa* WHEN COMPETING FOR A GROWTH-LIMITING SUPPLY OF K IN A MIXED CULTURE

K conc. (ppm)	Elodea					Draparnaldia					Mixed culture yield (g)
	Yield (g)		Prop. of yield (%)	K content (%)		Yield (g)		Prop. of yield (%)	K content (%)		
	Alone	+Drap.		Alone	+Drap.	Alone	+Elodea		Alone	+Elodea	
39	2.19	1.53	62	3.98	4.38	1.46	0.93	38	4.23	2.02	2.46
0.39	1.93	1.73	97	0.86	0.97	1.41	0.05	3	0.67	1.08	1.78

Table 17. GROWTH AND Ca CONTENT OF *Elodea occidentalis* AND *Draparnaldia plumosa* WHEN COMPETING FOR A GROWTH-LIMITING SUPPLY OF Ca IN A MIXED CULTURE

Ca conc. (ppm)	Elodea					Draparnaldia					Mixed culture yield (g)
	Yield (g)		Prop. of yield (%)	Ca content (%)		Yield (g)		Prop. of yield (%)	Ca content (%)		
	Alone	+Drap.		Alone	+Drap.	Alone	+Elodea		Alone	+Elodea	
32	1.90	1.59	69	0.78	0.71	1.48	0.72	31	0.20	1.49	2.31
0.8	0.09	0.08	5	0.46	0.51	1.28	1.52	95	0.05	0.04	1.60

Table 18. GROWTH AND Mg CONTENT OF *Elodea occidentalis* AND *Draparnaldia plumosa* WHEN COMPETING FOR A GROWTH-LIMITING SUPPLY OF Mg IN A MIXED CULTURE

Mg conc. (ppm)	*Elodea*					*Draparnaldia*					Mixed culture yield (g)
	Yield (g)		Prop. of yield (%)	Mg content (%)		Yield (g)		Prop. of yield (%)	Mg content (%)		
	Alone	+Drap.		Alone	+Drap.	Alone	+Elodea		Alone	+Elodea	
9.6	2.81	2.91	85	0.69	0.63	1.62	0.50	15	0.20	0.19	3.41
0.2	1.93	1.09	59	0.13	0.13	1.45	0.77	41	0.11	0.11	1.86

was 0.13%. This is close to the critical concentration of 0.10%. The Mg concentration in Draparnaldia was 0.10%, well below the critical concentration of 0.20%.

Two experiments were carried out in which two macrophytes (Elodea occidentalis and Myriophyllum spicatum) and the Draparnaldia were grown in mixed cultures. The data in Table 19 show the results from competition for a limited P supply, made available at 0.075 ppm. Again a somewhat superior competitive capacity of Draparnaldia is indicated because it represented 40% of the total yield. Myriophyllum (37% of the total yield) was somewhat more aggressive than Elodea (23% of the total).

The very low aggressiveness of Draparnaldia in K uptake at 0.39 ppm is supported by data in Table 20. Draparnaldia represented only 12% of the total yield. Myriophyllum was somewhat more successful in growth under low K than was Elodea. The former produced 53% of the total yield; Elodea represented only 35% of the total.

DISCUSSION

The primary goal of the studies reported was to demonstrate that the technique employed can be useful in characterizing the capacities of aquatic plants to compete for nutrients at the low concentrations found in lakes and streams. The validity of the technique seems justified by the results obtained, particularly the marked changes in the dominant organism when the organisms competed for different elements. For example, the green alga Draparnaldia was somewhat more successful than Elodea in competition for N and P at low concentrations. However, Draparnaldia was extremely ineffective in competition for K at 0.39 ppm. It made almost no growth. In contrast, Elodea made almost no growth when Ca was made available at a concentration of only 0.80 ppm.

Hopefully data obtained by the technique used can be applied in evaluating the role of nutrient supply and inorganic pollution in determining nuisance plant distribution. This of course will require

Table 19. GROWTH OF *Myriophyllum spicatum*, *Elodea occidentalis*, AND *Draparnaldia plumosa* WHEN COMPETING FOR A GROWTH-LIMITING SUPPLY OF P IN A MIXED CULTURE

P conc. (ppm)	*Myriophyllum*			*Elodea*			*Draparnaldia*			Mixed culture yield (g)
	Yield (g)		Prop. of yield (%)	Yield (g)		Prop. of yield (%)	Yield (g)		Prop. of yield (%)	
	Alone	+El. & Drap.		Alone	+Myr. & Drap.		Alone	+El. & Myr.		
6.2	1.06	0.73	33	1.07	0.50	23	0.79	0.95	44	2.18
0.075	0.86	0.52	37	0.99	0.32	23	0.61	0.56	40	1.40

Table 20. GROWTH OF *Myriophyllum spicatum*, *Elodea occidentalis*, AND *Draparnaldia plumosa* WHEN COMPETING FOR A GROWTH-LIMITING SUPPLY OF K IN A MIXED CULTURE

K conc. (ppm)	*Myriophyllum*			*Elodea*			*Draparnaldia*			Mixed culture yield (g)
	Yield (g)		Prop. of yield (%)	Yield (g)		Prop. of yield (%)	Yield (g)		Prop. of yield (%)	
	Alone	+El. & Drap.		Alone	+Myr. & Drap.		Alone	+El. & Myr.		
39	1.55	0.96	51	1.50	0.59	31	0.71	0.35	18	1.90
0.39	1.25	0.91	53	1.39	0.61	35	0.74	0.21	12	1.73

comparative studies with the various organisms observed to dominate in lakes or streams differing in fertility and nutrient composition. For example, several species of blue-green algae produce heavy blooms in eutrophic Wisconsin lakes and the green alga Cladophora is very abundant in areas of the Great Lakes following pollution.

The results obtained also are of interest in indicating that elements other than N and P may be of key importance in nutritional ecology. This is supported by the marked difference in response of Elodea and Draparnaldia to growth-limiting supplies of K and of Ca.

SECTION VIII

GROWTH OF *Elodea occidentalis* AT LOW CONCENTRATIONS OF INORGANIC NUTRIENTS MADE AVAILABLE IN SOLUTION-REPLACEMENT CULTURES

When grown in the laboratory, aquatic plants usually are cultured in small volumes of nutrient medium containing much higher concentrations of the essential elements than normally are present in lakes and streams. The high concentrations are necessary to provide amounts of the elements for high rates of yield from small volumes of solution. Data on the responses of aquatic organisms to element concentrations obtained under these conditions are rarely applicable to non-laboratory conditions.

Responses of organisms grown in the laboratory to the low concentrations of N, P, and other elements characteristic of natural waters can be measured by several techniques. These include continuous-flow cultures, the use of very large volumes of culture medium, and the frequent addition of a specific element to a culture to maintain a nearly constant, low concentration. The work to be reported was concerned with establishing the borderline concentration of N, P and each of the major element cations at which maximum rate of growth no longer could be maintained even though the total amount of an element made available to the *Elodea* was more than adequate.

EXPERIMENTAL PROCEDURES

Because of the large volumes of nutrient solution required, only two treatments, which were replicated, could be included in each experiment. In every experiment, one treatment always was the same control solution consisting of the medium described in Table 11 at one-quarter strength. The second treatment was the same as the control except one element was provided at a much lower concentration. Experiments were repeated with a progressively lower concentration of the element under study made available in each successive experiment until a concentration was established at which rate of absorption

was inadequate to produce yields equal to those in the control solution.

It was essential that the total amount of an element available to Elodea should be considerably in excess of the amount needed for the yield produced. At the lowest element concentrations studied, this required large volumes of solution. As a result, the lowest concentrations tested were determined by the solution volumes that could be provided conveniently.

To calculate the total amount of an element to be provided at low concentrations, it was recognized that about 500 mg of dry-weight Elodea would be produced in the 11-day experimental periods. On the basis of established Elodea critical concentrations an amount of the element was added to the cultures each day which would support a minimum of 100 mg of growth. For example, the Elodea critical P concentration is approximately 0.15%. To provide the required 0.15 mg of P at a concentration of 0.02 ppm it was necessary to add at least 8-liters of culture medium every 24 hours. Growth the last day of an 11-day period was calculated to be no more than 80 mg, if growth were continuous and exponential. Therefore, on that day the P made available to the Elodea was only 20% in excess of the requirement. On other days, additions of P relative to requirements were much greater.

Two types of culture apparatus were employed. The first experiments involved continuous-flow cultures using large test tubes as culture vessels. Sterile medium was pumped or allowed to flow through the culture vessels at a controlled and constant rate as in a chemostat. A more satisfactory apparatus, and the apparatus in which most of the experiments were carried out, utilized 6-liter Florence flasks fitted with bottom drains as culture vessels. At intervals controlled by a time clock, valves on the outlets opened and emptied the flask contents, except for the Elodea, and about 100 ml of culture medium. As soon as the vessels drained, the valves closed and fresh medium from pyrex carboys flowed into the flasks to a level controlled by a pyrex float valve. By adjusting the level of solution and the

length of time between drainings, any desired volume of solution could be provided.

All apparatus was thoroughly cleansed and sterilized prior to the initiation of an experiment. However, the large volumes of solution required made it impractical to sterilize the nutrient medium. Contamination did not prove to be a problem if the solutions were replaced at least once a day. When the culture vessels were drained, suspended contaminants as well as depleted medium were rapidly carried away.

The Elodea cultures were exposed to continuous fluorescent-tungsten light of approximately 900 foot-candles at approximately 25°C. Cultures were aerated with a 1% CO_2-air mixture. Harvested plants were dried at 65-70°C. Analysis for N, P, K and Ca were by the methods indicated in Section VII.

RESULTS

The results in Table 21 show that at 0.02 and 0.03 ppm P and at 0.30 ppm NO_3-N yields of Elodea were sharply and significantly reduced in comparison with yields in the control medium. Yield at 0.02 ppm P was reduced to 52% and at 0.30 ppm NO_3-N to 67% of control yields. The N and P analytical data support the conclusion that Elodea growth was limited by deficiencies of these two elements. Concentrations were very close to the critical values of 1.60% N in the second one-inch segment and 0.14% P in the same segment. Because of inadequate plant material, the analyses reported are of whole plants rather than of second one-inch segments.

The concentrations of Ca and K in infertile, soft water lakes can serve as reference values in evaluating the growth of Elodea at low concentrations of those two elements. In northern Wisconsin lakes with alkalinity values of only 4.0-6.0, Ca concentrations were as low as 0.5-1.5 ppm; K concentrations were 0.5 to 1.0 ppm. In an experiment not reported, yield was not significantly decreased when the Ca concentration was reduced to 0.80 ppm. However, yield was decreased sharply

Table 21. RESPONSE OF Elodea occidentalis TO VERY LOW CONCENTRATIONS OF ESSENTIAL ELEMENTS MADE AVAILABLE IN NUTRIENT SOLUTION REPLACEMENT CULTURES

Element varied	Element conc. (ppm)	Soln. volume (l)	Element added (mg)	Elodea yield		Element in Elodea		Added element absorbed (%)	t-test
				Dry wt. (mg)	Relative (%)	Conc. (%)	Wt. (mg)		
P	1.50	64.	96.00	528	100	1.11	5.86	6.1	
	0.03	64.	1.92	418	79	0.17	0.71	37.0	<.05
P	1.50	77.	115.50	331	100	1.20	3.97	3.4	
	0.02	77.	1.54	171	52	0.18	0.31	20.0	<.01
N	8.40	30.	252.00	409	100	4.25	17.38	6.9	
	0.30	30.	9.00	273	67	1.40	3.82	42.5	<.01
Ca	8.00	48.	384.00	698	100	0.58	4.05	1.1	
	0.37	48.	17.76	454	65	0.20	0.91	5.1	<.001
Ca	8.00	35.	280.00	318	100	0.56	1.78	0.6	
	0.17	35.	5.95	254	80	0.17	0.43	7.2	<.2
Ca	8.00	32.	256.00	254	100	0.52	1.32	0.5	
	0.035	32.	1.12	46	18	0.14	0.06	5.8	<.001
K	9.75	52.	507.00	482	100	4.03	19.28	3.8	
	0.10	52.	5.20	462	96	1.28	5.91	113.7[a]	>.50

[a]The 54 mg inoculum (...t weight) containing 5% K added 2.7 mg K to the experiment so the total uptake was closer to 85%.

at 0.37 ppm and 0.17 ppm Ca. There was almost no Elodea growth at 0.035 ppm. Plants that did grow were nearly black in color. At 0.37 ppm Ca, only 5.1% of the Ca made available in the 48-liters of nutrient solution was absorbed by the Elodea. The ineffective absorption of Ca at concentrations below approximately 0.50 ppm suggests that in lakes with very soft water, Ca supply may be a factor in determining which species are abundant.

The critical Ca concentration in the terminal one-inch of Elodea is 0.28%. In plants grown at 0.37 ppm Ca and less, even whole plant analyses (0.14-0.20%) were well below the critical concentration.

The absorption by Elodea of K made available at low concentrations contrasted sharply with the absorption of Ca at comparable concentrations. Even at 0.10 ppm K, there was no decrease in yield. Furthermore, the 1.28% K concentration in the Elodea was far above the 0.80% critical concentration, indicating the plants were absorbing sufficient K for normal growth. At 0.10 ppm, Elodea so effectively removed K from the nutrient solution that the indicated recovery was greater than the amount supplied. The additional K probably was derived from inoculum Elodea and from impurities in the large volumes of nutrient solution.

DISCUSSION

This study was primarily concerned with developing a technique for evaluating capacities to absorb nutrients at concentrations comparable to those in natural waters. The technique developed seemed very satisfactory for such studies with macrophytes and definitely superior to several types of continuous-flow cultures tested earlier on this project. The procedure would have to be modified to be effective with algae. In the apparatus used with Elodea, algae would be washed from culture vessels each time the nutrient solution was replaced.

Providing adequate total amounts of an element at low concentrations is a critical aspect of the procedure developed. The K results verify this was accomplished. There was no reduction in yield and

almost complete recovery of K supplied at only 0.10 ppm. Less efficient mechanisms for NO_3 and Ca uptake were indicated by sharply reduced yield with NO_3-N made available at 0.30 ppm and Ca at 0.37 ppm.

The data obtained seem of considerable interest in relation to the interpretation of chemical analyses of water samples. If nutrient concentrations in lakes and streams are reduced to the concentrations which gave decreased yields in these experiments, it is apparent that nuisance plant growths could be reduced for two reasons. First, amounts of the elements required for specific amounts of growth might not be available. Secondly, the concentrations might be reduced to levels at which rates of absorption could not keep pace with needs for growth, even though absolute amounts of the elements seemed adequate. In other words, it cannot be assumed that each unit of a nutrient is equally effective in promoting plant growth over the entire range of concentration present in lakes and streams. Data obtained by the procedure described would be useful in indicating concentrations at which this decreased effectiveness occurs for various elements. For example, the results correlate with the 0.30 ppm soluble N and 0.015 ppm P water concentrations at the start of the growing season considered critical for nuisance algae bloom production (Sawyer, 1947).

Data were obtained only with Elodea in this study. Application of this information to questions and problems relating to the importance of specific nutrients in controlling Elodea distribution in lakes must await comparative studies on the growth of other species of macrophytes and algae at very low concentrations of various elements. Application to nutritional ecology should be a major use of the information obtained.

The capacity of Elodea to obtain adequate K when made available at 0.10 ppm (considerably lower than concentrations in infertile lakes) correlates with the extreme aggressiveness of Elodea in K uptake when competing with a green alga for limited K in experiments described in Section VII.

SECTION IX

COMPARISONS OF RATES OF PHOSPHORUS AND RUBIDIUM UPTAKE BY SEVERAL MACROPHYTES AND ALGAE

This is a study to determine and compare the rates of P and K uptake in eight species of aquatic plants, to compare the uptake rates of Elodea occidentalis roots and shoots, and to predict the outcome of competition between species grown at low P or K levels from a comparison of uptake rates at those levels.

MATERIALS AND METHODS

Kinetic studies of PO_4 absorption were carried out with four species of aquatic flowering plants found in Wisconsin lakes: Elodea occidentalis, Ceratophyllum demersum, Myriophyllum spicatum, and Lemna minor. Four species of algae were also studied: two filamentous green algae, Draparnaldia plumosa and Stigeoclonium tenue, and two blue-green algae, Microcystis aeruginosa and Anabaena sp. Kinetic studies of Rb absorption involved the Elodea, Ceratophyllum, Myriophyllum, and Draparnaldia.

The macrophytes to be studied were cultured under continuous light of 500 f.c. in the nutrient solution described in Table 11. The four species of algae and the Lemna minor were grown (Table 22) in a modified Hughes, Gorham, and Zehnder medium (1958) under continuous light of 200 f.c. All species were grown at approximately 23°C and all except the Lemna and the algae were continuously aerated with a 1% CO_2 in air mixture. The plants were grown between 3 and 4 weeks, until enough material was present for an experiment. At the time the experiments were run, concentrations of P and K in all species were in excess of their respective critical levels.

For the experiments involving the macrophytes, except Lemna minor, the terminal two-inch segments of either roots or shoots were excised. Whole plants of Lemna were used. After being gently blotted on dry

Table 22. COMPOSITION OF THE SOLUTION USED FOR THE CULTURE OF ALGAE AND *Lemna minor*

Salt	Salt per 1. soln. (mg)	Element in soln. (ppm)
$NaNO_3$	496	N - 81.7
K_2HPO_4	39	K - 17.5
$MgSO_4 \cdot 7H_2O$	75	Ca - 9.8
$CaCl_2$	27	P - 6.9
$Na_2SiO_3 \cdot 5H_2O$	43	S - 9.8
Na_2CO_3	20	Mg - 7.5
EDTA	1	
Fe citrate	6	Fe - 1.12
Citric acid	6	
KCl[a]		Cl - 0.35
H_3BO_3[a]		B - 0.054
$MnSO_4 \cdot H_2O$[a]		Mn - 0.054
$ZnSO_4 \cdot 7H_2O$[a]		Zn - 0.026
$CuSO_4 \cdot 5H_2O$[a]		Cu - 0.006
$(NH_4)_6Mo_4O_{24} \cdot 4H_2O$[a]		Mo - 0.002
$CoCl_2$[b]		

[a]Trace element stock solutions were prepared at 5000x the concentration of the final solution. 1.2 ml of a solution of equal volumes of each of the trace element stock solutions was added to each liter of the final culture medium.

[b]$CoCl_2$ was added only to media for blue-green algae at a Co concentration of 0.001 ppm.

cheesecloth, approximately 0.4 g samples of plant material were weighed out and transferred to 60 ml of 0.5 mM $CaCl_2$ solution (Epstein, 1961) contained in 100 ml beakers. The beakers were then placed in a water bath maintained at 30°C for 15 minutes. Immediately before being placed in experimental solutions, plant samples were rinsed with two portions of 0.5 mM $CaCl_2$ at 30°C. At the start of an absorption period, the material was removed from the second rinse solution and placed in experimental solutions, which contained 0.5 mM $CaCl_2$ and were suitably labeled with either ^{32}P or ^{86}Rb. Solutions were continuously aerated during the uptake period. The ratio of solution volume to time (100 ml per 10 minute absorption period) was kept constant for all experiments (Andrew, 1966). The absorption period used when external concentrations were varied was 20 minutes.

At the end of the absorption period, experimental solutions were either sucked or poured off rapidly and the plant material was rinsed five times with an unlabeled KH_2PO_4 or KCl solution. The concentration of the rinse solution was at least ten times that of the labeled experimental solution and contained 0.5 mM $CaCl_2$. Plant material remained in the final rinse for 15 minutes, was then placed in aluminum bags and dried in a forced-draft oven at 60°C. After at least 48 hours, the dry weight of the material was determined.

Plant material being assayed for ^{32}P was ashed in a muffle furnace for 6 hours at 600°C after being pretreated as outlined by Bertramson (1942) for total P analysis. Material assayed for ^{86}Rb was ashed for 4 hours at 500°C. The ash, in both cases, was dissolved in 2 N HCl and quantitatively transferred to 10 ml volumetric flasks.

Two aliquots of each sample were counted in a Packard Tri-Carb liquid scintillation counter. Each P sample was counted for 1 minute and each Rb sample for 10 minutes using the ^{3}H channel. No scintillation solution was used.

The procedures in experiments involving algae were identical to the above except that green algae samples were removed from all solutions by filtration and blue-green algae samples were separated from

solutions by centrifugation.

THEORY

Phosphorus absorption, according to Hagen and Hopkins (1955), and cation absorption, according to Epstein and Hagen (1952), can be expressed by the following equations:

$$R + M \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RM \underset{k_4}{\overset{k_3}{\rightleftharpoons}} R' + M \qquad (1)$$

where R is a metabolically produced carrier, M is the ion being transported into the cell, RM is the carrier-ion complex, and k is the rate constant for each reaction. For the absorption of both P and K, k_4 is negligible. These reactions are analogous to the Michaelis-Menten (1913) theory of enzymatic reactions. An equation expressing the relationships in equation 1 is

$$(Vmax - v)[M]/v = Km \qquad (2)$$

where Vmax is the maximum rate of absorption at infinite substrate concentration, v is the observed rate of absorption at ion concentration M, and Km is the Michaelis constant or the ion concentration at $(\frac{1}{2})$Vmax.

Equation 2, as observed by Lineweaver and Burk (1934), can be written in a linear form:

$$1/v = Km/(Vmax[M]) + 1/Vmax \qquad (3)$$

If the process of absorption at a steady state involves a single first-order reaction, i.e., a single carrier mechanism, then a double-reciprocal plot of v and [M] will result in a straight line (Hagen and Hopkins, 1955; Hofstee, 1952). If more than one first-order reaction is involved, the double-reciprocal plot is a curvilinear line.

Equation 2 can be written in a second linear form (Hofstee, 1952):

$$Vmax = v + (v/[M]) \cdot Km \qquad (4)$$

Again, if only one first-order reaction is involved, the resulting plot of v against v/[M] is a straight line, but a curvilinear line results if two or more first-order reactions are involved. The Vmax of all reactions combined is given by the intercept with the ordinate, and the reaction components may be obtained graphically (Hofstee, 1952), so that Vmax and Km for each component may be calculated.

RESULTS

Check of Rb Labeling

Measurement of K absorption using radioactive tracers is difficult because the half-life of ^{42}K is very short (12.4 hours). In several systems, the absorption rate and mechanism of Rb uptake have been found to be identical to that of K (Epstein, 1961; Rains, 1968), and consequently, ^{86}Rb, with a half-life of 18.77 days, has been used as a tracer for K. In some systems, however, the uptake rates have been shown to be different (Jeschke, 1970), so a check of the validity of using ^{86}Rb as a tracer for K was made. For each species, cation uptake was measured in four different external solutions: 0.5 mM KCl, 0.5 mM RbCl, 0.01 mM KCl, and 0.01 mM RbCl. All solutions were labeled with ^{86}Rb. Results were calculated as mM cations absorbed and are presented in Table 23.

Table 23. COMPARISON OF K AND Rb UPTAKE RATES FROM EXTERNAL CONCENTRATIONS OF 0.5 mM KCl, 0.5 mM RbCl, 0.01 mM KCl, AND 0.01 mM RbCl

Plant	mM cation absorbed/g dry tissue wt/20 min ($x10^4$)			
	From 0.5 mM KCl (K,mM)	From 0.5 mM RbCl (Rb,mM)	From 0.01 mM KCl (K,mM)	From 0.01 mM RbCl (Rb,mM)
Myriophyllum shoots	150.47	57.82	0.69	0.74
Ceratophyllum shoots	142.14	167.73	7.72	5.80
Elodea roots	66.00	36.53	1.21	0.50
Elodea shoots	350.54	315.46	2.22	2.83

All solutions were labeled with ^{86}Rb; the concentration of $CaCl_2$ was 0.5 mM.

In three cases, Myriophyllum spicatum shoots at an external cation concentration of 0.5 mM and Elodea occidentalis roots at an external cation concentration of both 0.5 mM and 0.01 mM, Rb and K were not absorbed at the same rate. Results for Rb and K were comparable in all of the other trials. Because Rb could not be used as a tracer for K in all cases, experiments varying either time or external concentration were run with RbCl in the external solution rather than KCl. The uptake patterns obtained using RbCl can be applied, in most cases, to the uptake of K.

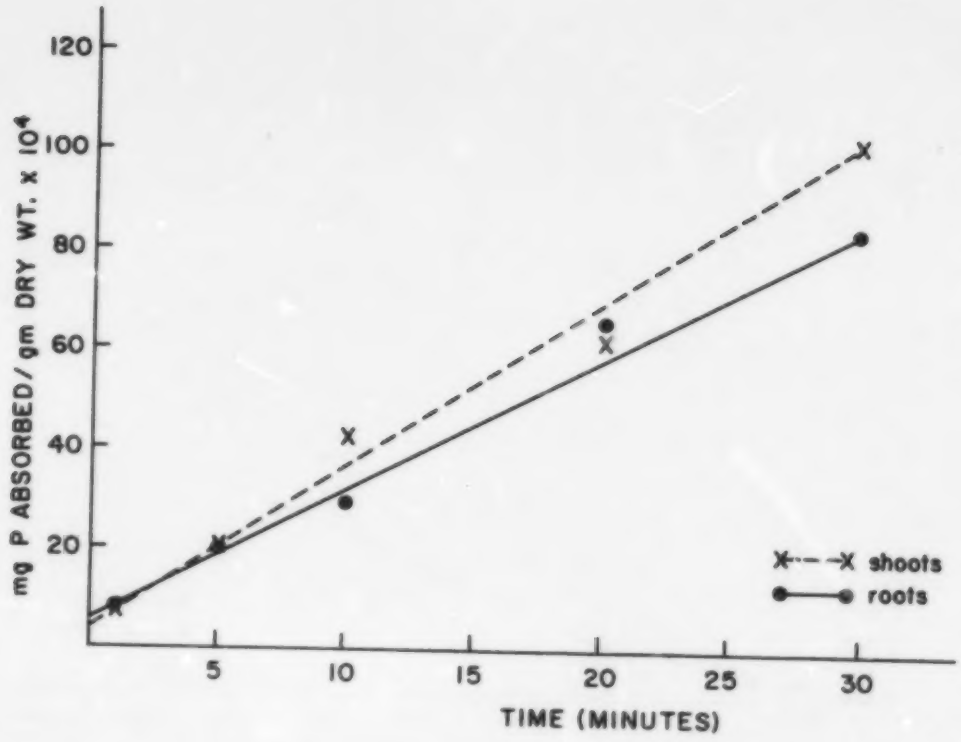

Figure 4. Relationship of phosphorus uptake to time in excised roots and shoots of Elodea occidentalis. Substrate concentration was 1×10^{-6} M KH_2PO_4 with $CaCl_2$ at 0.5 mM.

Time Experiments

The quantities of P absorbed by Elodea occidentalis roots and shoots are plotted against time in Figure 4 for time intervals between 1 and 30 minutes. The external P concentration was 0.031 ppm. The plot shows that steady-state conditions occurred for both the roots and shoots over a period of 30 minutes. The shoots showed a slightly higher rate of P uptake.

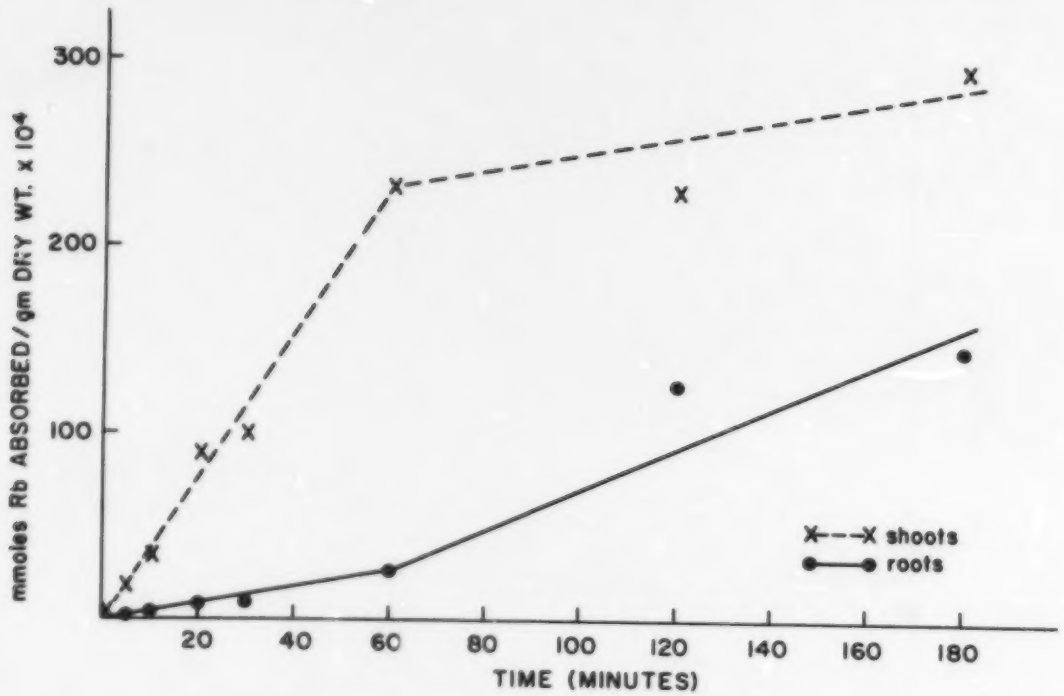

Figure 5. Relationship of rubidium uptake to time in excised roots and shoots of Elodea occidentalis. Substrate concentration was 0.5 mM RbCl with $CaCl_2$ at 0.5 mM.

A time plot for Ceratophyllum demersum shoots also was run but not reported. Steady-state conditions occurred over the first 20 minutes, but the uptake rate fell off at 30 minutes. A time plot for Stigeoclonium tenue showed steady-state conditions over a period of 60 minutes. Stigeoclonium showed an overall higher rate of P uptake than either of the macrophytes.

The plot of time against rate of Rb absorption for Elodea occidentalis roots and shoots is shown in Figure 5. The external RbCl concentration

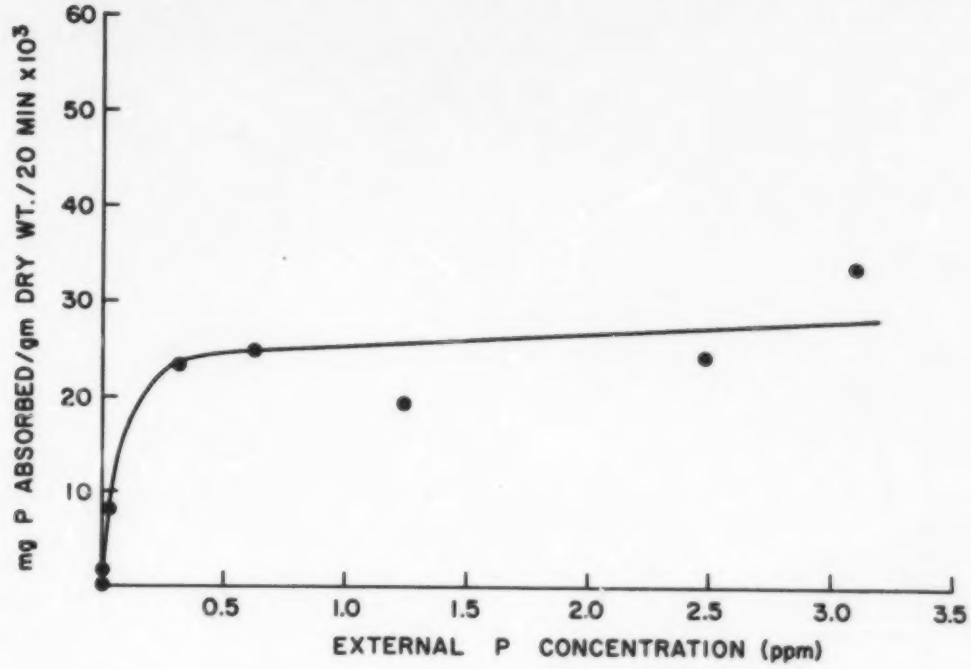

Figure 6. Relationship of rate of phosphorus uptake to external KH_2PO_4 concentration in excised roots of Elodea occidentalis. The concentration of $CaCl_2$ was 0.5 mM.

was 0.5 mM. Steady-state conditions occurred over the full 180 minutes for the roots, but only over the first 60 minutes for the shoots. Plots for Myriophyllum spicatum and Ceratophyllum demersum shoots resembled the plot for Elodea occidentalis shoots.

On the basis of these time experiments, it was concluded that steady-state conditions would occur, for the species examined, over at

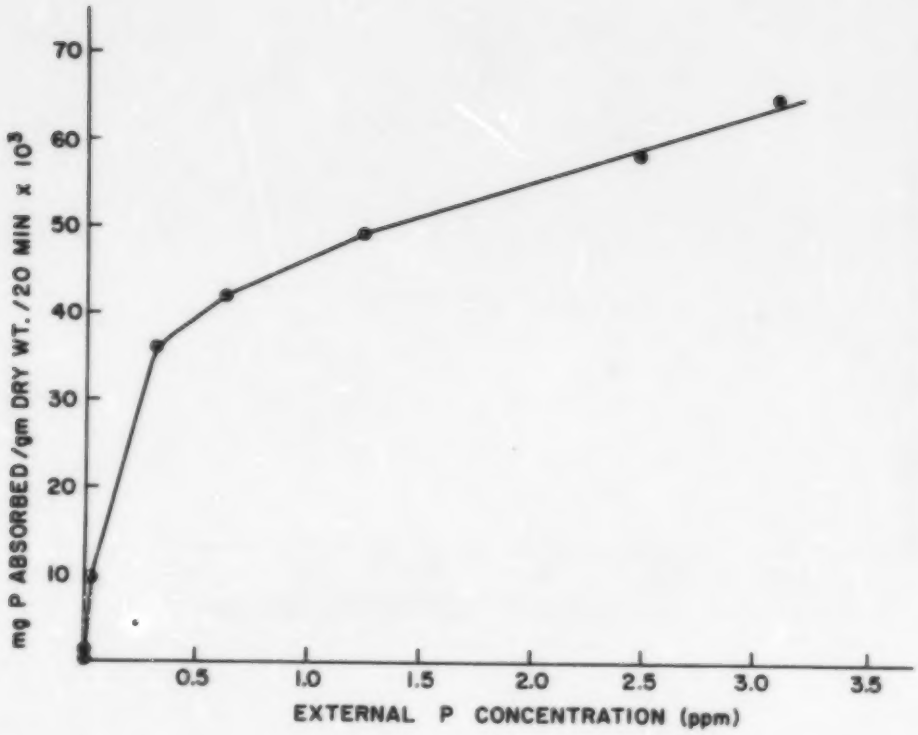

Figure 7. Relationship of rate of phosphorus uptake to external KH_2PO_4 concentration in excised shoots of Elodea occidentalis. The concentration of $CaCl_2$ was 0.5 mM.

least 20 minutes in experiments involving either P or Rb. Uptake periods of 20 minutes were used in all subsequent experiments.

Concentration Experiments - P

Rates of P uptake over a 0.00031 to 3.1 ppm concentration range were measured for all 8 species. Data for Elodea occidentalis roots and shoots, Draparnaldia plumosa, and Anabaena sp. are presented in Figures 6 through 9.

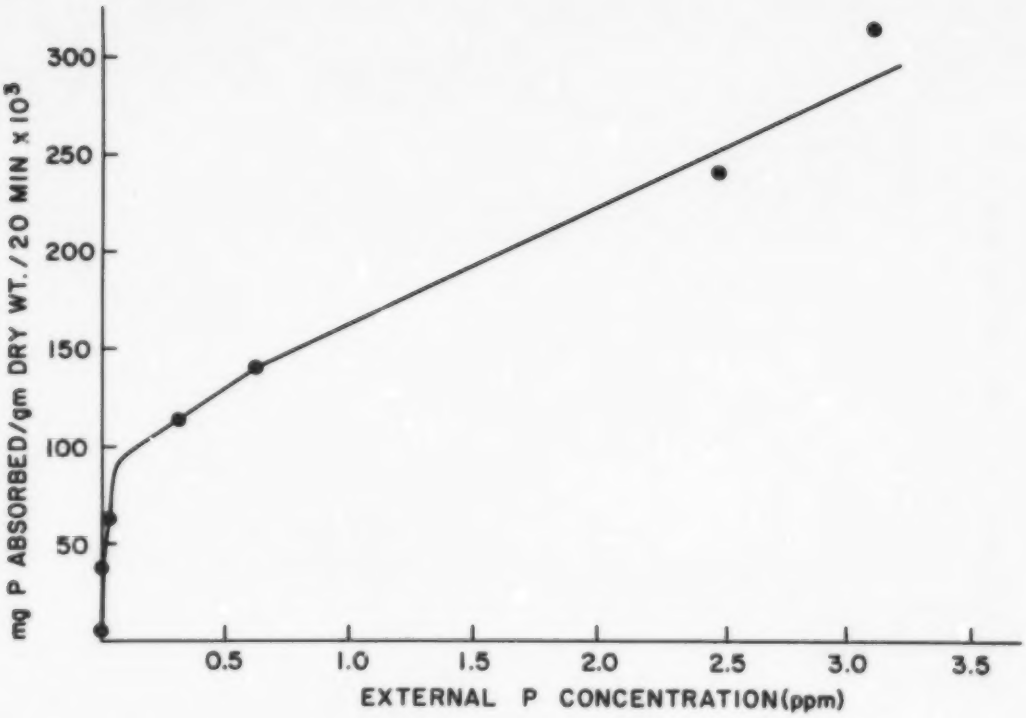

Figure 8. Relationship of rate of phosphorus uptake to external KH_2PO_4 concentration in Draparnaldia plumosa. The concentration of $CaCl_2$ was 0.5 mM.

In most species, rate of P absorption continued to increase even at the highest concentrations. Absorption did reach a maximum in Lemna minor, Anabaena sp., and Microcystis aeruginosa. Highest absorption rates occurred with Draparnaldia plumosa and Stigeoclonium tenue, the two species of green algae tested. The relationship

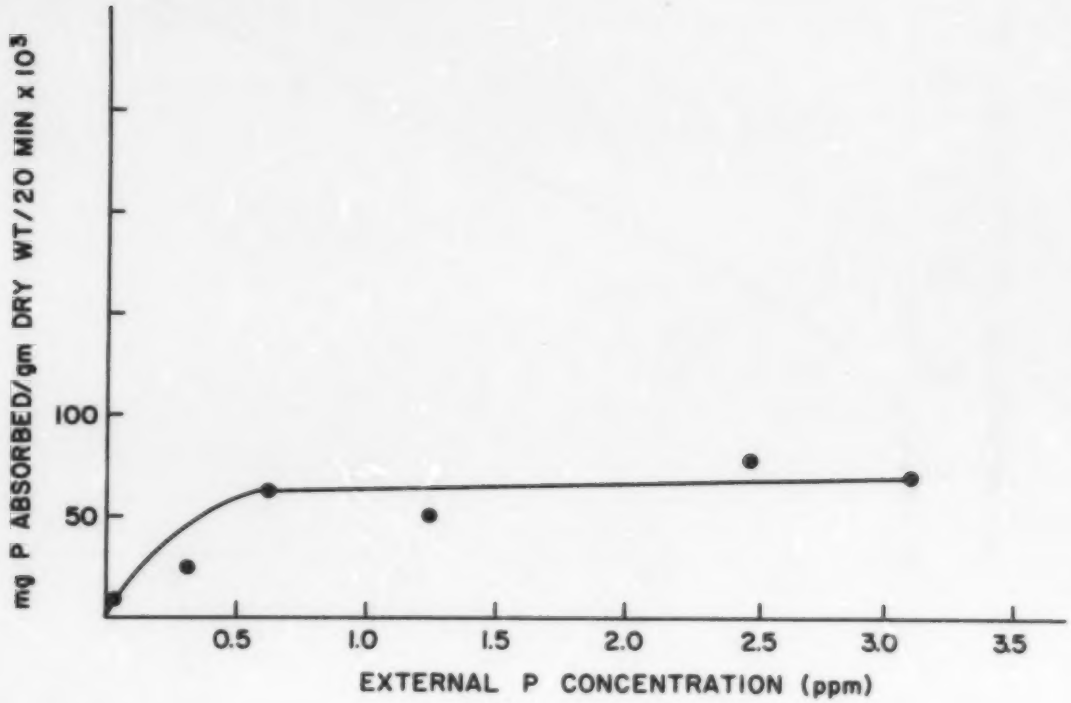

Figure 9. Relationship of rate of phosphorus uptake to external KH_2PO_4 concentration in Anabaena sp. The concentration of $CaCl_2$ was 0.5 mM.

between species regarding absorption rates was not constant from one external concentration to another.

When double-reciprocal plots of absorption against external concentration are made (Lineweaver and Burk, 1934) for Elodea occidentalis shoots and Draparnaldia plumosa, the resulting lines are curvilinear, not straight, as shown in Figure 10 and 11. The plots for all eight species tested were curvilinear which suggests that more than one first-order reaction is involved in P absorption (Hagen and Hopkins, 1955).

Figures 12 and 13 represent plots of P absorption against absorption

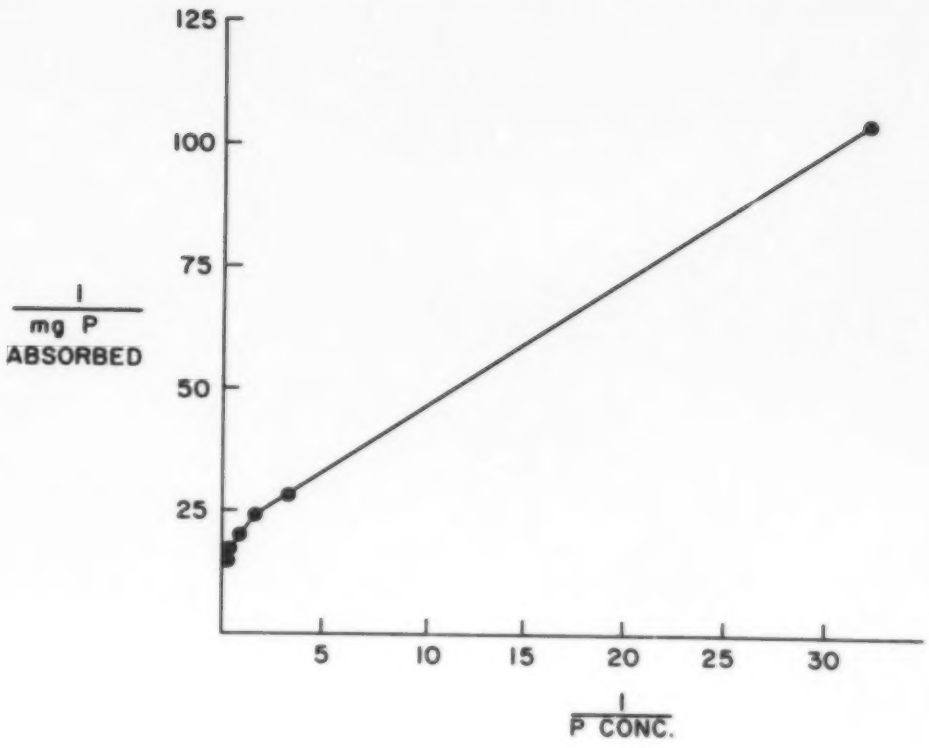

Figure 10. Double-reciprocal plot of rate of phosphorus uptake in excised shoots of Elodea occidentalis in relation to external concentration.

over P concentration, as originally done by Hofstee (1952) for enzymatic reactions. As in the double-reciprocal plots, the resulting lines are curvilinear rather than straight. They were curvilinear for all eight species. This also suggests that more than one first-order reaction is involved in P absorption.

Using graphical methods, these curvilinear plots were resolved into their two straight line components, mechanism "a" and mechanism "b". Mechanism "a" is a low affinity site and operates principally at high external P concentrations, while mechanism "b" is a high

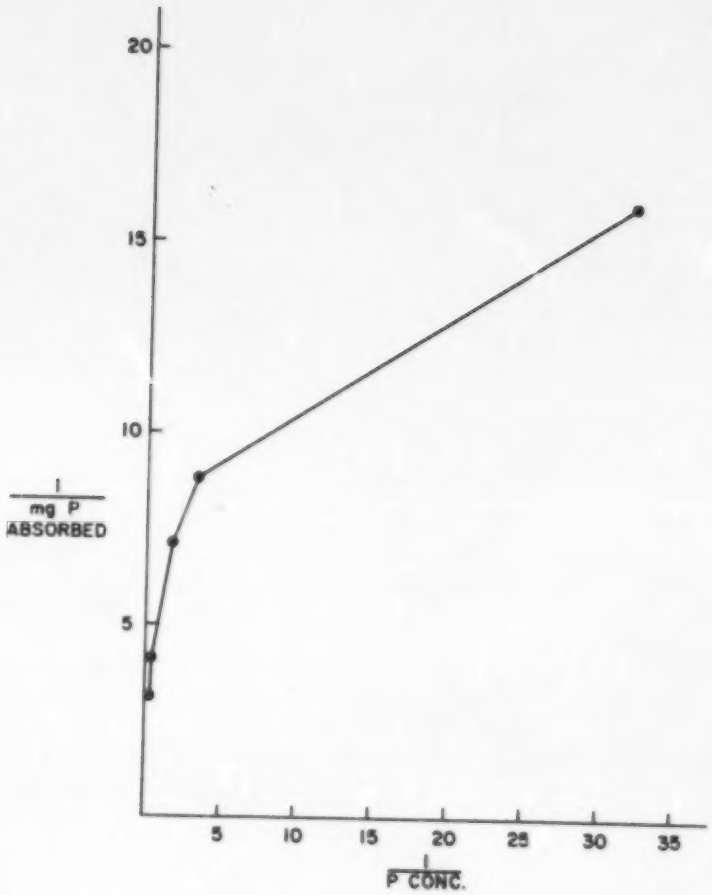

Figure 11. Double-reciprocal plot of rate of phosphorus uptake in Draparnaldia plumosa in relation to external concentration.

affinity site and operates at low external P concentrations, although both operate simultaneously. From the straight line components, values for Vmax and Km for mechanism "a" and mechanism "b" were calculated (Table 24).

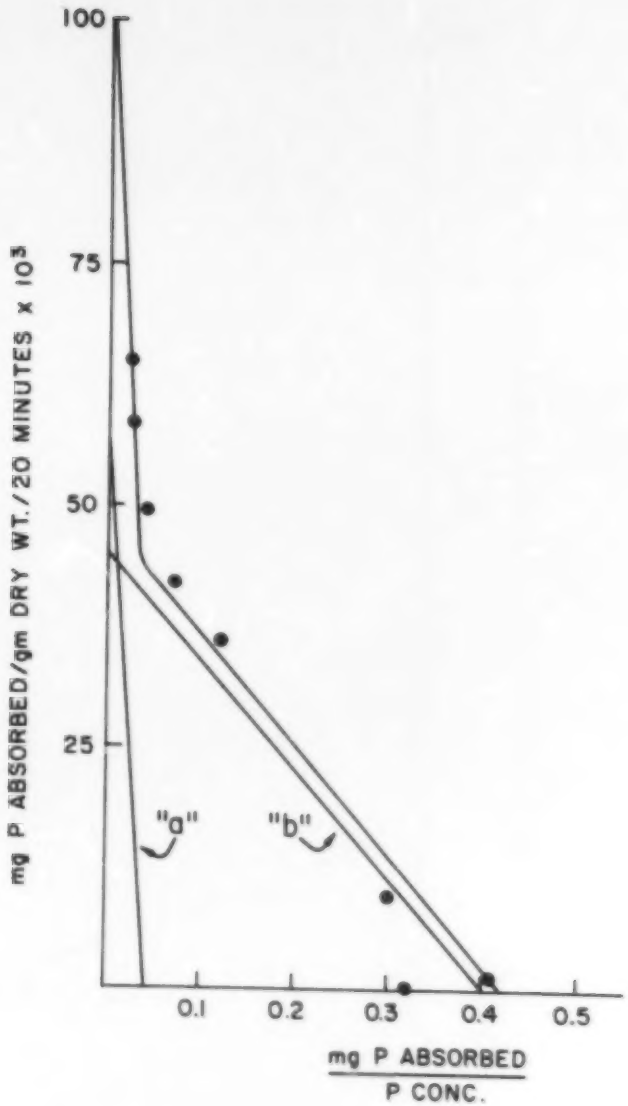

Figure 12. Relationship between rate of phosphorus uptake and phosphorus uptake/external phosphorus concentration for excised shoots of Elodea occidentalis.

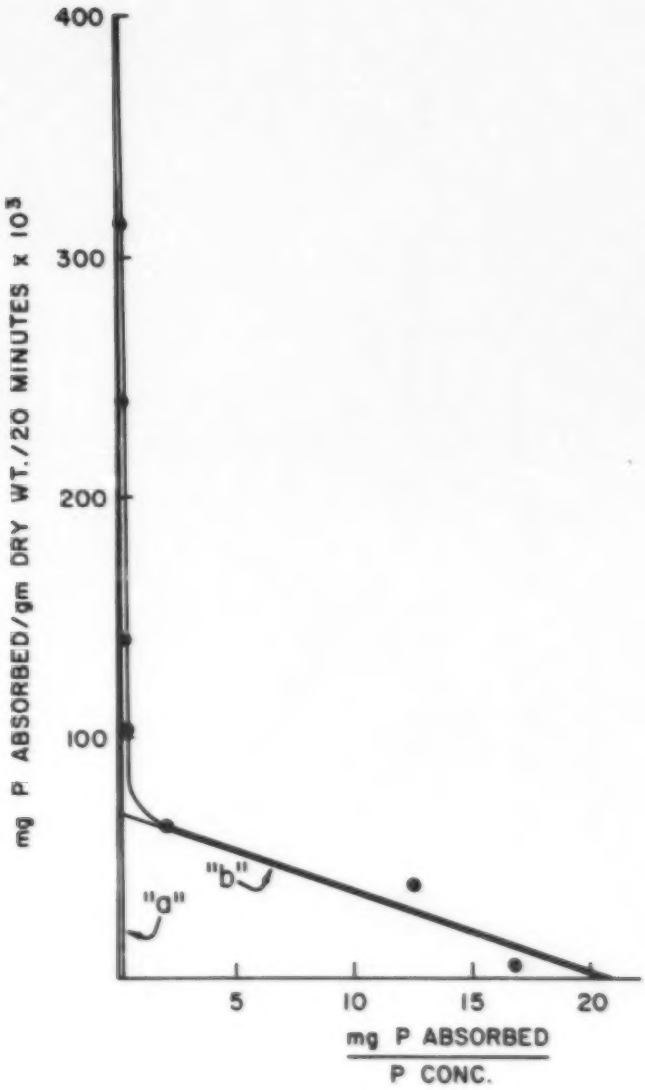

Figure 13. Relationship between rate of phosphorus uptake and phosphorus uptake/external phosphorus concentration for Draparnaldia plumosa.

Table 24. THE APPARENT Vmax AND Km OF THE TWO CARRIERS INVOLVED IN P UPTAKE IN EIGHT SPECIES OF AQUATIC PLANTS

Species	$Vmax_a$	$Vmax_b$	Km_a	Km_b
	mg P abs./g dry wt./20 min ($x10^3$)		ppm	
Elodea occidentalis roots	34	8	1.12	0.012
Elodea occidentalis shoots	55	45	1.29	0.011
Ceratophyllum demersum	67	1	0.37	0.0023
Myriophyllum spicatum	43	1	1.15	0.00045
Lemna minor	60	30	1.34	0.123
Draparnaldia plumosa	330	70	1.65	0.0036
Stigeoclonium tenue	100	70	0.27	0.015
Microcystis aeruginosa	185	2	2.47	0.004
Anabaena sp.	100	10	2.07	0.024

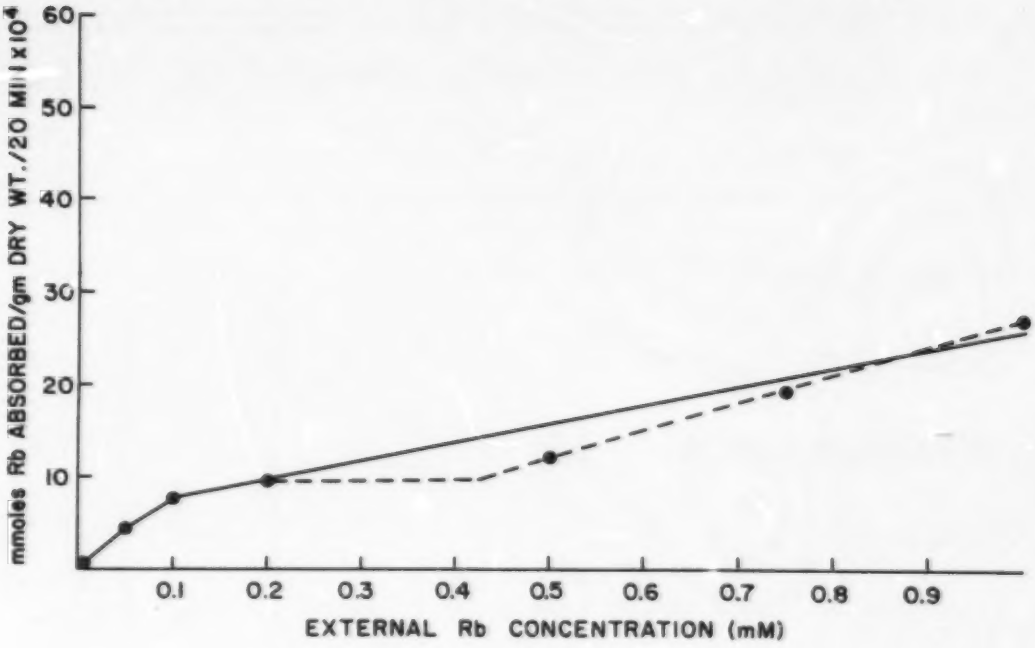

Figure 14. Two possible relationships between rate of rubidium uptake and external RbCl concentration in excised roots of Elodea occidentalis. The concentration of $CaCl_2$ was 0.5 mM.

Figures 14 and 15 show the relationship between external Rb concentration and rate of Rb absorption in Elodea occidentalis roots and shoots. The external Rb concentration varied from 0.002 to 1.0 mM.

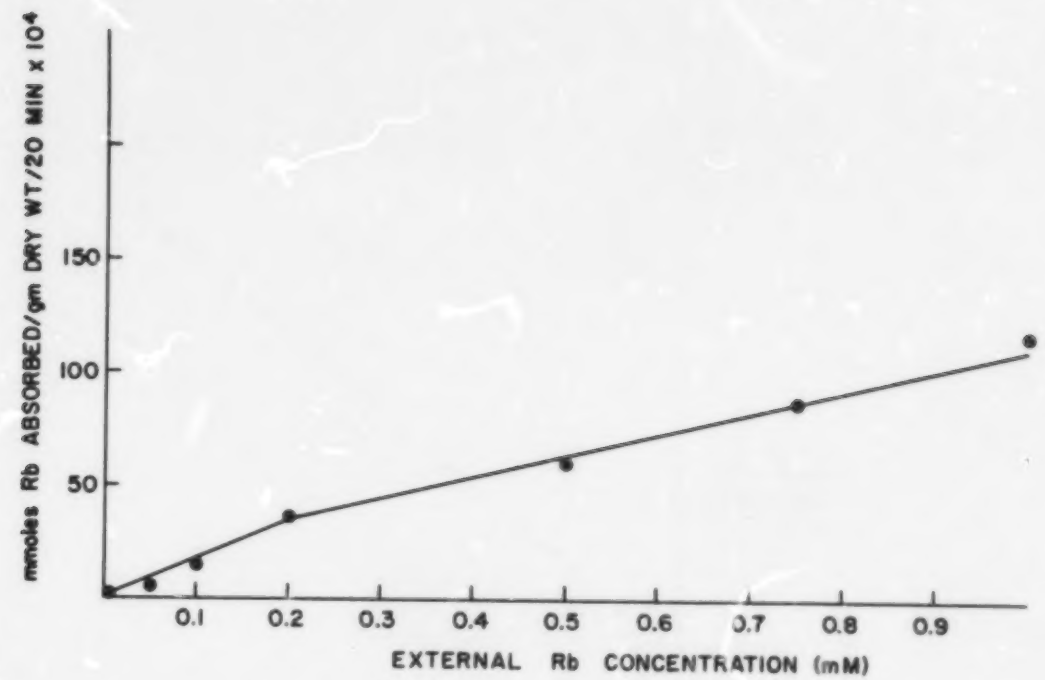

Figure 15. Relationship of rate of rubidium uptake to external RbCl concentration in excised shoots of Elodea occidentalis. The concentration of $CaCl_2$ was 0.5 mM.

When double-reciprocal plots of Rb absorption rate against external concentration were made, as shown in Figures 16 and 17, for roots and shoots of Elodea occidentalis the resulting line for the roots was curvilinear; for the shoots, the line was straight. In unreported

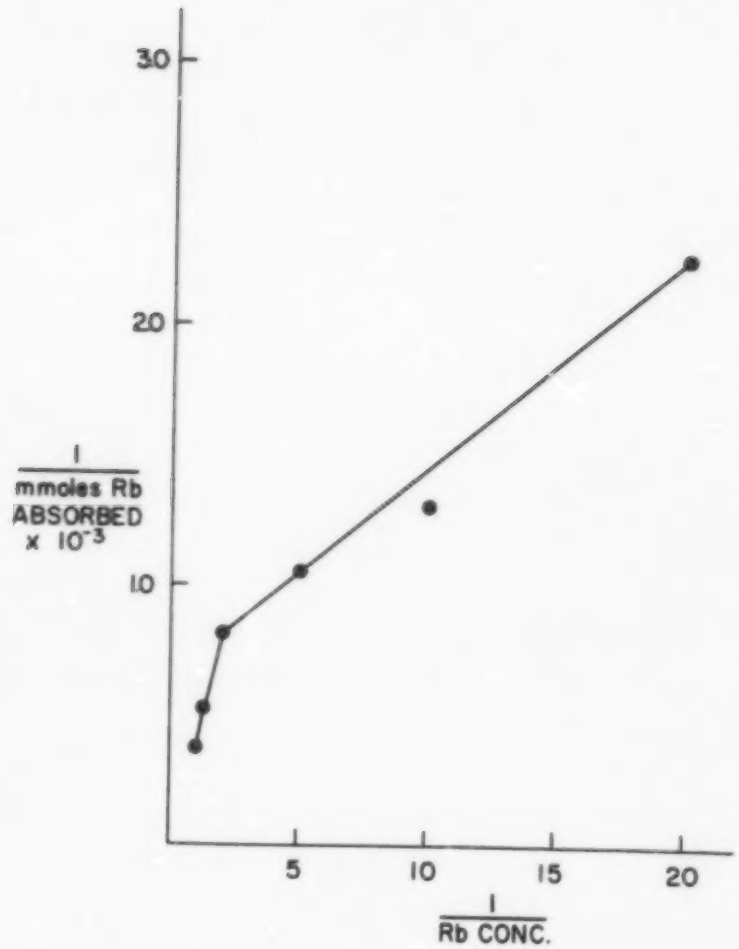

Figure 16. Double-reciprocal plot of rubidium uptake in excised roots of Elodea occidentalis.

experiments, a straight line also was obtained for Ceratophyllum demersum shoots and a curvilinear line for Myriophyllum spicatu[illegible] shoots and the alga Draparnaldia plumosa. These somewhat limited data suggest that, within the range of concentrations teste[illegible], one

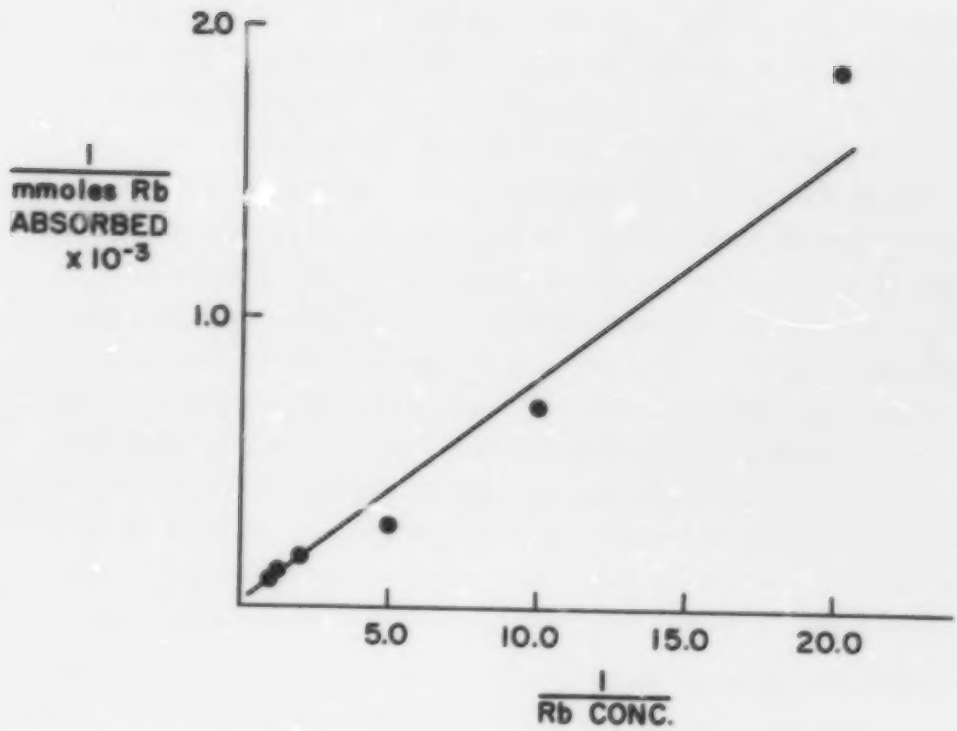

Figure 17. Double-reciprocal plot of rubidium uptake in excised shoots of Elodea occidentalis.

mechanism is involved in Rb and K uptake by Elodea and Ceratophyllum shoots and two or more mechanisms in the other species. Data were considered insufficient to compute Vmax and Km values.

DISCUSSION

Whether submerged aquatic plants absorb ions mainly from the substrate or from the surrounding water has long been a topic of controversy,

largely because of the lack of direct experimental evidence for ion absorption by the roots of aquatic macrophytes (Sculthorpe, 1967). Most of the studies on ion absorption in aquatic macrophytes have been with shoots and leaves. Recent work in several laboratories on both freshwater (Bristowe and Whitcombe, 1971; DeMarte and Hartman, 1974) and marine (McRoy and Barsdate, 1970) angiosperms has shown that the roots of some aquatic macrophytes do absorb ions and that these ions are then translocated to the shoot. The results in Figures 4, 5, 6, and 14 indicate that roots of Elodea occidentalis absorb both P and Rb. At low P and Rb concentrations, under aerobic conditions, the rates for both roots and shoots are comparable, but at high P concentrations, rates for the shoots are higher than for the roots. At least under aerobic conditions, the relative contribution of the roots and shoots to the P or K nutrition of a whole plant would depend on the root/shoot ratio of the plant and on the ion concentrations in the substrate and surrounding water.

Kinetic studies of ion uptake by roots have led to the concept that for many nutrient elements two apparent sites or mechanisms are involved in active ion uptake (Epstein, 1972). Most of the studies have been with crop plants, such as barley and corn; very little work has been with aquatic macrophytes, although dual mechanisms for K (Jeschke, 1970), PO_4, and SO_4 (Jeschke and Simonis, 1965) have been reported in Elodea densa leaves. The results in the present experiments indicate that two mechanisms are involved in P absorption and are similar to those found in barley (Hagen and Hopkins, 1955), corn (Carter and Lathwell, 1967), alfalfa and millet (Noggle and Fried, 1960). In all cases, the two mechanisms operate simultaneously, although one mechanism (site b) has a higher affinity for P than the other. Both $Vmax_a$ and $Vmax_b$ were higher for the aquatic plants than for barley (Hagen and Hopkins, 1955) and corn (Carter and Lathwell, 1967) roots and both Km_a and Km_b were lower in the aquatic plants. The lower the Km value, the higher the affinity of the ion carrier for the ion, so both uptake mechanisms have a higher affinity in the aquatic plants for P than their counterparts in barley and corn.

Rate of ion uptake is one primary quantitative nutritional difference between plant species. The results obtained indicate that the P and Rb uptake rates of the various aquatic plants and plant parts do differ. On the basis of these differences, certain species might be predicted to outcompete others in nutrient deficient environments. For example, based on uptake rates at 0.0031 ppm P, under conditions of low P supply Draparnaldia plumosa would be expected to outcompete the other seven species tested. Myriophyllum spicatum would be the least effective in competition for P. The indicated uptake rates for all eight organisms at 0.0031 ppm P was: Draparnaldia > Stigeoclonium > Elodea > Microcystis > Anabaena > Ceratophyllum > Lemna > Myriophyllum.

At an external concentration of 0.05 mM Rb, the relative uptake rates for the four organisms tested were: Draparnaldia > Ceratophyllum > Elodea > Myriophyllum. This relationship also would be the predicted outcome of competition between these organisms under limited K supply. The Rb uptake rate for Ceratophyllum demersum was 8x greater than for Myriophyllum spicatum. This might explain the much higher concentrations of K in Ceratophyllum than in Myriophyllum collected from Lake Wingra (Section VI). The higher Rb uptake rate in Draparnaldia than in Elodea does not, however, correlate with the much greater aggressiveness of Elodea when competing with Draparnaldia for a growth-limiting K supply as described in Section VII. Further study will be required to establish which procedure more accurately reflects competition for nutrients under field conditions. Until confirming information is available, there seems justification for attaching greater reliability to a procedure in which effective competition for an element is actually reflected in plant yield.

SECTION X

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